ANNUAL REPORT
OF
PROGRAM ACTIVITIES

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Annual Report of Program Activities
National Institute of Allergy and Infectious Diseases
January - December 1959

Office of the Director

The accomplishments of this Institute, as in previous years, have commanded the respect of the scientific community in regard to the intramural research program as well as the extramural investigations support awarded to scientists here and abroad. We wish to express concern, however, that we have not been able to secure support for the Institute's research training grant activities which would allow them to be exploited fully. It is our sincere belief that failure to recognize the urgency for expanding training programs in microbiology and immunology can work only to the detriment of nationwide scientific progress in the basic health sciences.

The following items, recounted in detail in subsequent pages, merit special mention:

1. The National Advisory Allergy and Infectious Diseases Committee on Standardization of Allergens, activated during the last calendar year, has developed a program which promises very substantial contributions to both diagnosis and treatment of allergic conditions, grave problems for many years. A conference of experts held in June developed a definite program for the Committee. Three special groups were established, one of which will study chemical and physical characteristics of the active allergen fraction, the second will develop biological testing systems, and the third group will be concerned with clinical trials. A supply of standard ragweed pollen was procured for distribution to participating scientists so that all work on standardization will have a common starting point from which valid comparisons can be made.

2. The Surgeon General's Advisory Committee on Influenza Research requested the Council to support a program of special influenza research. To this end, $175,000 was earmarked in fiscal years 1960 and 1961 to support the program recommended by the Committee. A special review and approval procedure has been developed to insure expeditious handling of grants for these investigations.

3. The Virology and Rickettsiology Study Section recommended to Council that there should be a Committee charged with the responsibility of establishing criteria of classification of agents as adenoviruses. A committee was established under the aegis of the Council to do this and to set up standards to insure

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production of uniform reagents. The Committee will also evaluate methods of activation and classification, and will consider new agents for inclusion as adenoviruses. Although the work of the Committee has international implications, it has been established to serve only the United States at this time.

4. Directors of training grants programs throughout the country have called to the attention of NIH the need for stabilization of their programs. To this end, during 1959 the Institute took the necessary steps to adjust the beginning dates of all active training grants to assure support to the grantee institution for an ensuing academic year. This makes it possible to award traineeships and to employ the faculty necessary to insure continuity of training programs.

5. The NAAIDC recognizes that because of the nature of the program of the Institute it will always need to support a certain amount of research outside the United States. It is equally aware that a national position in this consideration has not been firmly established and has, therefore, made it a matter of Council policy to support only those foreign applications of unusual merit and uniqueness. Criteria for acceptance include unusual ability of investigators and geographical incidence of diseases of special interest. It was voted that the dollar value of these special grants supported should not exceed 3½ percent of the appropriation available for research projects.

6. Dr. Vernon Knight, a clinical research scientist of outstanding ability, joined NIAID this year to direct the programs of clinical investigation of the Institute. Under his leadership the program is showing wholesome growth. Sound plans for the future have been made and we have every assurance that this will be one of the more productive clinical research centers in this country.

7. As reported in the previous two years, the Middle America Research Unit has engaged in an active research program in Panama and other Central American countries as provided in the original agreements drawn between the Canal Zone Government, the Department of Defense, and the Public Health Service. A Board of scientists evaluated this program in October of this year. They recommended that the laboratory be established as a permanent field station of the National Institute of Allergy and Infectious Diseases. On the basis of this recommendation, steps are being taken to negotiate a continuing contract among those concerned to insure the future growth and development of the Middle America Research Unit.

8. Responsive to expanding research horizons, changing objectives, and the growing scientific interests of the intra-

mural staff over the past several years, the organizational structure of the laboratories was revamped. The philosophy behind the change was to create small laboratories which could have more sharply defined research objectives and direction by scientists of demonstrated research competence and administrative skill. To achieve this, eleven basic laboratories were created from six. The wisdom of this move has been demonstrated in the ease with which the transition was effected and the rapid development of research programs within these units.

9. Special mention is made of an honor bestowed upon one of the senior scientists of the Institute. Dr. Jules Freund received a Lasker Award in Medical Science, presented at the annual meeting of the American Public Health Association in Atlantic City in October 1959. This is a most fitting recognition of the outstanding contributions which have been made by this distinguished member of our Institute.

Annual Report of Program Activities

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Extramural Programs

Despite laudable gains in the prevention and treatment of communicable diseases, the problems caused by microbiologic and parasitic infections still rank near the top in any study of disabling illnesses. Equally disturbing is the knowledge that half our population suffers from varying degrees of hypersensitivity. Recognizing the significance of this situation, the Institute has maintained a balanced research and training program focused on the basic nature of disease-producing organisms and allergenic substances, together with related problems of transmission, host resistance, preventive measures, and specific therapy.

Research Grants

The distribution of research funds in support of the Institute's objectives is indicated in Tables I and II. Data regarding the review and approval of research grants are given in Table III.

The Council initiated and maintained special emphasis in areas where particular needs exist. Concerned by the lack of legal standards for the potency and purity of allergenic extracts, it established a permanent Committee on Standardization of Allergens to look into the entire problem, including research needs. Supported by a grant of $20,000, the Committee in June held a scientific symposium to develop a program for action. As an initial step the Committee purchased a supply of standard ragweed pollen from which participating scientists will prepare allergenic fractions. These will be submitted to other experts for immunochemical identification, serological and biological testing, and finally clinical testing.

Recognizing the aura of ignorance that still surrounds the nature, distribution and control of the sarcoid state, the Council awarded funds to the National Academy of Sciences to support an International Conference on Sarcoidosis in June, 1960. Chief purposes of the conference will be to stimulate research on the epidemiology and etiology of the disease, and to seek general agreement on a definition of sarcoidosis and criteria for its diagnosis.

Early this year the Council focused attention on special needs in two other areas through partial sponsorship of research conferences on (1) "The Biology of Pleuropneumonia-like Organisms" and (2) "Cystic Fibrosis". In further support of investigations on cystic fibrosis the Council endorsed supplementation of a study being made

by the Children's Bureau to determine the actual prevalence of the disease in the United States. In all, the Institute now sponsors 23 projects for $346,369 concerning the occurrence and infectious aspects of this disease.

Antibiotic-resistant staphylococci continue to present grave problems, especially in hospitals. Mindful of this, the Council has recommended an increasing number of research projects in this area. At present the Institute supports 84 grants for a total of $1,403,994. In addition to numerous basic investigations on bacterial pathogenicity, host resistance, and related problems of fundamental importance, significant studies on transmission in hospitals are being sponsored. One such project concerns the design of hospital nurseries in relation to the spread of organisms; another is a large-scale coordinated study by five medical school hospitals and the National Research Council to evaluate ultraviolet irradiation in the prevention of postoperative wound infection.

Noting that the intensity of research on influenza tends to ebb and flow with the retreat and advance of epidemics, the Council has stimulated development of a sustained program of inter-epidemic research. Continued support has been provided for the Public Health Service Advisory Committee on Influenza Research. Moreover, the Council voted to reserve for each of fiscal years 1960 and 1961 the sum of $175,000 for "Special Grants" to be awarded on the recommendation of a Subcommittee of Investigators and approval of the Council. In addition, the Institute is sponsoring a symposium to be held in February, 1960, on the 1957-58 Asian influenza pandemic. This conference will emphasize deficiencies in present knowledge and thus will suggest areas for intensive research.

The Council took cognizance of the urgent need for standardized criteria and methods for the identification and characterization of agents of the adenovirus group, and for the production of standard reagents to be used in diagnosis and research. The Council urged establishment and support of a committee under its auspices to define the problem and secure the participation of qualified laboratories in meeting the need.

In consonance with the Institute's long-standing interest in tropical medicine and related fields, support has been recommended for a forthcoming symposium on research needs in this area. This conference, scheduled to be held in New Orleans during April, 1960, will bring together a group of selected authorities to delineate the specific aspects which should be emphasized in future research on tropical diseases and environmental medicine.

The Council has felt it both justified and desirable to foster research at the place and by the people most likely to produce significant results. Thus it has awarded research grants to scientists at foreign institutions whenever a highly competent investigator proposed a project which for geographical or other reasons could best be done abroad, or when the investigator was so outstanding that his contribution would have general scientific significance. On this basis approximately 38 foreign grants totaling $460,000 dollars have been supported during the year. The Council recently recommended that 3½ per cent of the Institute's extramural research grant appropriation be reserved for foreign grants. The amount thus reserved for fiscal year 1960 is $755,755.

Current appropriations for this and five other Institutes of the National Institutes of Health include funds for the planning, negotiating, and initial support of clinical research centers spanning the interests of these six Institutes. Such centers would be established in a few large medical research institutions throughout the United States. Concurring in the need for a centralized review mechanism, our Council recommended to the Surgeon General that responsibility for the review of applications in this program be delegated to the National Advisory Health Council.

In conformity with the policy developed to satisfy the President's first criterion (regarding scientific excellence), the Council at its November meeting gave individual reconsideration to applications recommended for approval at the June meeting but falling within the lowest ten per cent of the priority list for each study section. Special consideration also was given to the corresponding portion of new proposals being reviewed for the first time. In confirming its previous recommendations, the Council made it a matter of record that the policy of the National Advisory Allergy and Infectious Diseases Council has consistently been such as to cause this category of applications to be brought to its attention for special consideration and close scrutiny before recommendations or dispositions were made.

Training Grants

During the present year the Division of General Medical Sciences assumed responsibility for research training in those areas of microbiology primarily related to organisms as biological models rather than as agents of disease. Accordingly, 16 grants for basic research training in microbiology were transferred to the Division of General Medical Sciences, without transfer of funds. Thus the Institute may now concentrate its training programs along lines which emphasize its disease-oriented interests in allergic and infectious disorders.

Table IV indicates the distribution of training grant funds in support of the Institute's objectives. Table V gives data regarding review and approval of training grant applications during the calendar year.

Rapid growth in the number of applications received for graduate training grants, together with reorientation of the program as just described, has made it desirable to divide the original Training Grant Committee into two panels. These will be known as the Allergy and Immunology Training Grant Committee and the Infectious Diseases and Tropical Medicine Training Grant Committee.

To bring the fiscal year of training grants into phase with the normal academic year of training institutions, all training grants have been or soon will be pro-rated and financed on a basis of adjusted project period dates corresponding to the government fiscal year. This has resulted in the payment, from a single year's appropriation, of 18 or more months support for many grants. Accordingly, during fiscal year 1960 there will be insufficient funds to activate all new applications recommended for approval.

The Council is aware that unique opportunities exist in certain foreign institutions which have provided outstanding training to Americans as well as their own nationals. In recognition of this, one training grant has been recommended for approval at an institution in Canada with the proviso that stipends from the grant be awarded to American citizens only. The Council has expressed its desire that at least half the funds awarded through all training grants be devoted to stipends and other direct trainee support such as tuition, travel expenses, and consumable supplies.

The Council and Training Grant Committee have continued to plan for the support of a few centers of outstanding competence for the training of scientists in tropical medicine and related disciplines. Emphasis is being placed on extremely careful planning in securing rapport with foreign collaborating establishments. Because of many important implications over and beyond technical and scientific considerations, action on pending proposals has been deferred until the June 1960 meeting to allow time for the development of better defined programs.

Research Fellowships

Under a plan approved by the Surgeon General, the Administration of all active and pending predoctoral research fellowships has been transferred to the Division of General Medical Sciences. Consequently, a sub-allocation of $446,100 from the current appropriation of the Institute has been made available for this purpose.

Table VI presents data regarding the review and approval of fellowships during the calendar year, and the distribution of awards by areas of study. The fellowship program has followed the trend established last year, with increased emphasis on awards at the postdoctoral and special levels to produce increasing numbers of highly competent independent investigators. Approximately one third of the applications recommended for approval could not be awarded because of insufficient funds.

TABLE I

RESEARCH GRANTS PROGRAM (NIAID)

(FISCAL YEARS)

Area of Support	'57 Appropriation* $8,212,000		'58 Appropriation* $10,824,000		'59 Appropriation* $14,880,000		'60 Appropriation*** $21,593,000	
	No.	Amount	No.	Amount	No.	Amount	No. (Estimated)	Amount
Allergy	71	$1,091,324	92	$1,339,584	107	$1,550,824	140	$2,186,000
Immunology	57	669,522	65	776,143	77	1,033,864	123	1,855,000
Bacteriology	164	1,847,267	213	2,594,636	280	3,791,288	388	5,563,000
Virology	104	1,736,694	146	2,701,257	173	3,426,721	243	4,771,000
All Others (Antibiotics, Arthropods, Biochemistry, Epidemiology, Genetics, Mycology, Parasitology, etc.)	351	2,947,130	407	3,752,420	492	5,085,583	584	6,465,000
Grand Total	747	$8,291,937**	923	$11,164,240**	1,129	$14,888,280**	1,478	$20,840,000

* The '56 and '57 appropriations included $147,000 for Gorgas Memorial Laboratory, and the '57 appropriation also included $21,000 for Russian Translation Service; the '58 and '59 appropriations include $150,000 for Gorgas and $18,000 and $39,000 respectively for Russian Translation Service. In addition, $442,000 was deducted from the '59 appropriation to cover NIAID salary increases.

** Excess listed over appropriation represents extensions without additional funds and grants, paid from previous year fiscal funds, which will terminate during current fiscal year.

*** The '60 appropriation includes $500,000 for metabolic (clinical) centers, $150,000 for Gorgas Memorial Laboratory, $44,000 for Russian Translation Service, $1,000 for the CDC Diagnostic Reagents Program, and $43,000 for the Children's Bureau project on cystic fibrosis.

TABLE II -- RESEARCH GRANTS PROGRAM (NIAID) SPECIAL AREAS [1]

Area of Support	Fiscal Year 1957		Fiscal Year 1958		Fiscal Year 1959		Fiscal Year 1960	
	No.	Amount	No.	Amount	No.	Amount	No.	Amount (Estimated)
1. Acute Respiratory Disease [2]	42	$ 739,844	58	$1,153,109	71	$1,428,586	102	$1,980,000
2. Cystic Fibrosis	4	35,700	4	36,511	23	382,539	22	346,000
3. Parasitology and Tropical Medicine:								
Sub-total	258	2,146,234	281	2,539,916	331	3,229,304	394	4,354,000
Parasitology:								
Helminths	59	459,136	67	592,196	78	696,375	98	929,000
Protozoa	67	565,640	64	566,072	76	762,524	83	885,000
Entomology (Arthropods):	57	419,006	60	496,189	75	653,187	91	877,000
Arthropod-borne Viral Diseases:								
Dengue	1	14,641	2	37,732	4	69,339	4	89,000
Encephalitis	3	68,392	5	99,955	7	135,046	11	298,000
Yellow Fever	-	- -	1	32,545	1	27,500	1	25,000
Non-Parasitic Tropical Diseases of Importance:								
Cholera	1	10,350	1	7,360	2	19,165	4	41,000
Leprosy	4	72,824	7	78,709	7	106,297	8	140,000
Salmonellosis	4	33,235	6	65,225	8	96,586	13	133,000
Shigellosis	5	51,416	2	20,126	3	70,726	2	85,000
Mycology	57	451,594	66	543,807	70	592,559	79	852,000
4. Staphylococci:	10	102,699	19	189,573	59	1,002,566	84	1,483,000
5. Tuberculosis and related areas:	34	524,744	44	694,979	73	995,618	89	1,340,000
6. Enteroviruses [3] (excluding poliomyelitis)	-	- -	-	- -	9	166,730	17	347,000
7. Urinary Tract Infections (Pyelonephritis) [3]	-	- -	-	- -	5	108,378	12	191,000

1/ Included in areas listed in Table I.

2/ Includes Airborne Allergy, Common Cold, Influenza, Periodontic, Pneumococcal, Q Fever and Viral Pneumonia.

3/ Not separately listed under Table III.

TABLE III

RESEARCH GRANTS REVIEWED DURING CALENDAR YEAR 1959

	Received		Approved	
March	No.	Amount	No.	Amount
New	182	$2,643,270	115	$1,427,836
Continuations	86	1,133,857	71	873,586
	268	$3,777,127	186	$2,301,422
June				
New	228	$3,565,614	162	$2,510,025
Continuations	968*	13,615,473	962*	13,465,151
	1,196	$17,181,087	1,124	$15,975,176

* Includes continuation grants for which previously recommended support was reaffirmed. (897 for $12,792,730).

November				
New	232	$3,530,306	140	$1,873,976
Continuations	64	831,395	56	536,003
	296	$4,361,701	196	$2,409,979

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TABLE IV

TRAINING GRANT PROGRAM (NIAID)

(FISCAL YEARS)

	'58 Appropriation $650,000 1/		'59 Appropriation $1,787,000		'60 Appropriation $3,621,000	
Discipline	No.	Amount	No.	Amount	No. (Estimated)	Amount
Allergy and Immunology	12	$254,145	19	$525,203	29	$1,112,000
Infectious Diseases 2/	7	159,377	23	793,830	28	1,200,000
Tropical Medicine and Parasitology	8	151,392	18	439,668	29	1,259,000
	27	$564,914 3/	60	$1,758,701 3/	86	$3,571,000 3/

1/ Includes $70,000 sub-allocated to Division of Research Grants for General Research Training Grant Program.

2/ Infectious Diseases included only mycology and rickettsiology in Fiscal Year 1958.

3/ Chairman's grants are not included in these figures.

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TABLE V

TRAINING GRANTS REVIEWED DURING CALENDAR YEAR 1959 1/

March	Received No.	Received Amount	Approved No.	Approved Amount
New	15	$ 634,042	10	$ 473,277
Continuations	1	11,880	1	11,880
	16	$ 645,922	11	$ 485,157
June				
New	31	$ 993,505	17	$ 440,665
Continuations	59	1,627,580*	59	1,627,580*
	90	$2,621,085	76	$2,068,245
November				
New	19	$ 981,139	14	$ 459,313**
Continuations	0	0	0	0
	19	$ 981,139	14	$ 459,313

* (Includes continuation grants for which previously recommended support was reaffirmed).

** (Includes $99,743 for three grants subsequently transferred to DMH)

1/ Amounts do not include $50,000 approved as a Chairman's Grant, nor $948,700 required to adjust training grant programs to the July 1 beginning dates.

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TABLE VI

RESEARCH FELLOWSHIPS REVIEWED DURING CALENDAR YEAR 1959

	Predoctoral		Postdoctoral		Special	
	No.	Amount	No.	Amount	No.	Amount
Received (New)	171	$598,500	98	$647,000	18	$144,000
(Continuations)	41	143,500	6	39,000	1	8,000
	212	$742,000	104	$686,000	19	$152,000
Recommended for Approval: (New)	127	$409,021	96	$549,179	13	$ 88,530
Inactivated: (New)	51	$165,858	19	$110,120	3	$ 25,176
Awarded: (New)	76	$237,603	77	$439,059	10	$ 71,354
(Continuations)*	68	150,056	15	85,142	1	5,434
	144	$387,659	92	$524,201	11	$ 76,788

Number of Awards by Categories and Levels

Category	Predoctoral	Postdoctoral	Special
Antibiotics	3	-	-
Entomology	5	-	-
Bacteriology	11	9	3
Biochemistry	15	22	-
Chemotherapy	4	1	-
Rapid Identification	-	-	-
Epidemiology	-	-	-
Mycology	4	-	1
Genetics	3	4	1
Helminthology	1	5	1
Immunology	3	9	1
Allergy	3	8	-
Protozoology	6	1	-
Public Health & Sanitation	6	-	1
Physiology	3	1	-
Rickettsiology	-	-	-
Spirochetology	1	2	-
Taxonomy	1	-	-
Virology	7	15	2
	76	77	10

*Includes applications received before January 1958

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ACCOMPLISHMENT HIGHLIGHTS

A growing list of significant research accomplishments reported by grantees of this Institute attests to the steady development of the extramural programs. The published reports represent not only additions to our knowledge of specific diseases, but also important discoveries in the basic processes which govern pathogenesis. Without these fundamental studies there would be no firm foundation for the fruitful expansion of new research horizons. The broad range of interests and responsibilities within the extramural programs are partially indicated by the representative examples given below.

Noteworthy advances have recently been achieved by grantees in the diagnosis and treatment of allergies. Scientists at the California Institute of Technology studying the chemical and physical properties of house dust allergens report the preparation of a purified fraction that gives characteristic skin reactions. This finding may provide new information for improving and standardizing the house dust extracts widely used in diagnosis and hyposensitization of persons allergic to this ubiquitous material.

A grantee at Temple University School of Medicine found that a citrus extract is more effective than corticoids in inhibiting capillary permeability in rabbits. The phenomenon is seen as an index of anti-inflammatory potential. The investigator points out significant clinical implications in his findings that capillary permeability, a basic reaction in the inflammatory process, is suppressed in rabbits receiving a water-soluble citrus bioflavonoid. The bioflavonoid appears to display a larger range of inhibition potentialities than either cortisone or ACTH. The clinical usefulness of this water-soluble agent is being investigated.

At the University of California grantees sensitized stressed mice to bovine plasma albumen and administered a shocking antigen dose. They found that stress increased the resistance of the mice to anaphylactic shock. Immediately following periods of stress, the mice are less susceptible to passive anaphylactic shock than their unstressed counterparts. Having observed a similar decrease in susceptibility after the administration of cortisone, the investigators theorized that increased adrenal cortical activity may be responsible. They conclude that whatever the mechanism, this change in susceptibility provides additional evidence for the influence of environmental stress on host response to infections and allergic disease.

The Council has been troubled by the lack of factual data on the incidence of cystic fibrosis. It therefore endorsed an appropriate investigation by the Children's Bureau. The first evidence on the occurrence and distribution of the disease has been compiled; additional studies are in progress.

One of our grantees at Harvard University reviewed the effectiveness of antibacterial drugs over the past 24 years. He found that the early reduction in mortality is now more than offset by increasing deaths as a result of drug resistance and conversion of relatively harmless bacteria to highly pathogenic agents. The favorable impact of the antibacterial drugs in reducing the number of deaths from pneumococcal and the hemolytic streptococcal infections has actually been overshadowed by an increase in deaths from infections by Staphylococcus aureus, and enterococci, and certain coliforms which only rarely caused fatalities before the era of antibacterial drugs. There were nearly four times as many cases of Staphylococcus bacteremia reported in 1957 as in 1935, and more than twice as many as in 1947.

A team of grantees associated with Harvard, Boston, and Tufts Universities reported a spectrum of findings in a study of fatalities associated with Asian influenza in the Boston area. Staphylococcal pneumonias were frequently observed. The investigators found the community to be a source of antibiotic-resistant staphylococcus strains. Influenzal and post-influenzal pneumonias were the more common and severe complications. The investigators suggest that in times of epidemic influenza, and in areas where staphylococcal infections are highly prevalent, cases presenting signs usually associated with fatal influenza should be treated promptly as bacterial, and particularly staphylococcal, pneumonias. The most striking characteristic of the organisms associated with both the fatal and the nonfatal cases was the exceptionally high incidence of strains resistant to multiple antibiotics.

Basic investigations regarding the fundamental nature of parasitism and the relationships existing between parasites and their hosts form a solid core of this Institute's grant-supported program. An exciting development in this area has been reported very recently by grantees at Johns Hopkins University working with certain protozoan parasites of birds, closely related to species parasitic in man. These scientists found that the ability to produce disease could be transferred to normally harmless strains of the parasite by treating them with cell-free filtrates of the culture medium in which pathogenic strains had been grown. This report of the genetic transformation of parasitic protozoa represents a significant advance in the field of parasitology and of biology in general.

Grantees at the University of Miami School of Medicine have found that dermatomycoses are responsive to systemic treatment with the new drug, griseofulvin. The mycoses caused by various species of dermatophytes showed a uniformly favorable response to oral therapy with griseofulvin. Infections of long duration, up to 60 years in one case, seemed to respond as readily as those present for

a few weeks or a month. Ringworm of the skin usually cleared within one to two weeks. Athlete's foot improved in one to two weeks but required a month or longer to clear. Ringworm of the scalp improved in two to three weeks but sometimes required more prolonged treatment. No acquired resistance to the drug was noted and toxicity was extremely low. The grantees conclude that "the systemic treatment of superficial fungus infection in man at last seems a near reality."

One approach to the problem of immunization against tuberculosis is being exploited by grantees who have reported success in protecting guinea pigs against tubercle bacilli through the inhalation of weakened or attenuated organisms of the BCG strain. Initial trials of this method with humans appear to have been successful. The investigators, who are associated with the National Jewish Hospital and Colorado School of Medicine at Denver, Colorado, suggest that the airborne method should also be attempted in other types of respiratory infections.

Other studies on vaccination by use of live pathogens are being conducted by grantees at the Wistar Institute and Children's Hospital of Philadelphia. These scientists gave attenuated poliomyelitis virus vaccine orally to infants less than six months of age. They claim to have demonstrated effective antibody response to all strains except one, without harmful side effects. As a result of their studies the grantees believe that vaccination against poliomyelitis with orally administered, living, attenuated viruses is entirely practical in infants over two months of age if three-week intervals are allowed between feedings of the three types of virus. These investigators contend it also is possible to vaccinate successfully most infants over two months of age--even on the first day of life.

The chance occurrence of a measles epidemic at a California mental hospital led to re-examination of an earlier vaccine study in which the investigators, grantees at UCLA School of Medicine, attempted to determine whether distemper vaccine might reduce the incidence of respiratory infections. The results suggest that a considerable degree of protection may be afforded against measles by inoculation with live canine-distemper virus. The scientists report a three-fold reduction in the attack rate of measles associated with use of the canine-distemper virus.

THE ANNUAL REPORT OF THE SCIENTIFIC DIRECTOR
The National Institute of Allergy and Infectious Diseases
Calendar Year 1959

by

Dr. Dorland J. Davis

The pursuit of new knowledge and the employment of new methods for studying infectious and allergic diseases have accelerated during the year 1959. This has been evident not only in the etiologic, epidemiologic, and clinical aspects of disease, but also in the basic understanding of how cells react to infection. The exploration of infectious processes has always contributed to an understanding of human diseases, whether actually caused by microorganisms or not. Host parasite relationships have frequently been models for the reaction of the living cell and tissue to noxious agents. Many of our techniques of investigating human diseases have originated in studies of parasitism. For instance, the methods of immunology now applied to non-infectious processes were derived in part from the study of immunity to infectious agents. Conversely, it is becoming increasingly apparent that infectious agents may have latent or heretofore hidden influences on human diseases not thought to be infectious. For example, new knowledge of viruses has led to the supposition that some or possibly all abnormal growth of cells may in a direct or remote way be related to infectious viruses which enter the cell and take control of it. These and other concepts are emerging as new advances demonstrate the extensive influences of microbial agents on complex forms of life such as the mammalian host.

The aim of the research program of this Institute is to conduct selective and influential investigations within the areas of its mission. The resources available to scientists here in Bethesda, carry with them the responsibility that they be used most efficiently in studies directed toward the most important and significant questions. These are not always evident and require experienced insight to recognize. The ability of the researcher to ask the right questions, to devise proper experimental methods, evaluate results properly, and report clearly, determines the difference between investigations of high quality and those of little importance. The development of effective research projects in large part derives from the

imagination and persistence of the individual scientist who develops his own approach and research objectives. If such an investigator works in a favorable environment and atmosphere with adequate support, influential new knowledge usually results. It is essential therefore, that the institutional organization be so structured that opportunities for original thought and unconventional methods are not only possible, but are encouraged.

Within the last few years increased emphasis has been placed upon the selection of staff members who will bring originality to their investigations. This is in part accomplished by selecting persons with special skills in allied medical sciences from leading research centers. Another principle which has been endorsed and will be followed more vigorously in the future, is to permit certain Institute scientists to study in other research centers for a period of at least a year. This opportunity is aimed particularly at the younger scientist who has given evidence of research competence, but has not yet become involved in a large comprehensive program.

Another phase of development of research potential has been in the experience offered to young medical graduates who serve their National Selective Service obligation in the Public Health Service. These men spend two and sometimes three years in clinical studies or in full-time laboratory investigations with a senior experienced scientist. They now form, and will continue to form a valuable group from which to recruit permanent staff members for NIAID, or for positions in teaching institutions.

In addition, there are a number of guest workers from domestic and foreign institutions working with NIAID scientists for periods of several months to a year. In effect, a significant segment of the activities of the Institute are involved in advanced experience in research by carefully selected young scientists.

In line with the concept that the organization of the NIAID should fit its research mission and the individuals conducting it, a new administrative structure of the Institute was effected in 1959. This recognized the development of several new programs around key researchers and broadened objectives of several project areas. The Laboratories of this Institute now number eleven, including two with major activities located outside Bethesda, the Rocky Mountain Laboratory at

Hamilton, Montana, and the Middle America Research Unit in the Panama Canal Zone. Studies in tropical disease formerly unified under the direction of Dr. Willard Wright were separated into the Laboratory of Parasite Chemotherapy, Laboratory of Parasitic Diseases, the Laboratory of Germfree Animal Research, and a portion of the Laboratory of Tropical Virology. The Laboratory of Cell Biology and the Laboratory of Biology of Viruses, both with an outstanding senior investigator as Chief, are now separate from their former organizational place in the Laboratory of Infectious Diseases. The Laboratory of Clinical Investigation under a newly appointed Clinical Director, has continued its studies of clinical diseases in patients and efforts made to strengthen its contacts with the nonclinical laboratories. The continuance of former investigations and initiation of new ones promise well for the results of clinical research in the future.

The Board of Scientific Counselors met in July at the Rocky Mountain Laboratory to consider the research programs of that center. In the fall the Board met in Bethesda to review the activities of the Laboratory of Clinical Investigations and their relationship to the total research effort of the Institute. The members of the Board have been quick to comprehend Institute resources, and potential for future investigations, and likewise the special problems involved. Their advice has been constructive and pertinent. Individual members of the Board have been helpful in advising on special research projects within their area of experience, and in assisting in the recruitment of both junior and senior staff members.

A review of the accomplishments of the last year, indicates that Institute research is moving into new areas both scientifically and geographically. The investigations at MARU have not only permitted new projects in Central America such as those on the encephalitides, poliomylitis and influenza in tropical areas, but also has stimulated an interest in other special problems of warm climates. This has resulted in plans for clinical studies of schistosomiasis in Puerto Rico, and the possibility of importation to the Clinical Center of patients with tropical diseases.

Advancement has occurred in studies of tumor viruses and viruses causing tumors both in laboratory reared animals and also in animals in their natural state. Studies in association with the National Institute of Neurological Diseases and Blindness

of the occurrence of human infections during the perinatal period have extended greatly the opportunity for investigation of viral infections in adults and children. This is consistent with the past efforts of this Institute to study disease as it actually occurs in nature, rather than to depend chiefly upon laboratory models of natural phenomena. The future will see a continuation of this approach, a strengthening of the clinical investigation program using the unparalleled facilities of the Clinical Center, and an emphasis on basic phenomena occurring in mammalian cells either infected or uninfected with micro-organisms.

The broad interests of Institute scientists in infectious and allergic diseases, and fundamental cellular and subcellular processes underlying the disease state are illustrated in the following summary of our research activities.

During 1959 the Laboratory of Infectious Diseases continued as its primary interest investigations of the role of viruses, bacteria and fungi as causes of disease.

For instance, the research program of the viral and epidemiological sections are almost wholly integrated. Their closely interwoven research programs are designed to define the behavior and importance of viruses (respiratory viruses, enteroviruses, and cancer viruses) in their natural hosts, observed in certain natural environments. Thus, studies of respiratory diseases and other undifferentiated illnesses were continued in two pediatric groups - at Junior Village and at Children's Hospital, and in groups of adults, including several installations of the U. S. Navy and at the University of Maryland. The role of viruses in respiratory and intestinal diseases of cattle were observed on farms, feedlots and in experimental pens at the Universities of Maryland and Illinois. The potencies and possible effectiveness of respiratory virus vaccines were examined in a preliminary manner in relation to both human and animal respiratory illness, and broad sero-epidemiologic studies of the prevalence of many viruses in various age and economic groups in various parts of the world were initiated.

During 1959 viruses as a cause of tumors and cancer in animals developed into a major research interest. At first studies were focused on the development and evaluation of new techniques for demonstrating and detecting tumor viruses in experimental mouse colonies and chicken flocks. In July, 1959 attention turned to the occurrence of mouse and avian tumor viruses in natural populations.

RESPIRATORY VIRUS AND ENTEROVIRUS STUDIES

The National Health Survey 1957-1958 revealed once again and with greater force than ever the magnitude of Acute Respiratory Diseases in the U. S. A. It was estimated that several hundred million such illnesses had occurred and confirmed previous impressions developed in several independent studies about their greater prevalence in children. Consequently, studies of this essentially unsolved problem were pursued with undiminished vigor.

The Junior Village orphanage study, now in its fifth year, continued to serve magnificently in its dual role as (1) a source of basic and essentially new information on the total viral and bacterial experiences of the urban infant, and (2) serving as a focal point for a number of more specific studies on the behavior and possible prevention of certain of the apparently more important agents.

Analysis of the overall experiences, now completed, for three years continued to show that an average of 25 percent of the infants suffered a new acute febrile illness (exclusive of measles and chickenpox) each week; and that approximately 30 percent of them acquired a new identifiable viral or bacterial infection during the same interval. A total of over 1933 separate infections with viruses belonging to 53 different serotypes and 718 infections with presumed bacterial pathogens were observed. Crude estimates indicate that 40 percent to 45 percent of the more severe illnesses observed in the orphanage infants could be attributed to known agents, most of them viral. Statistical analysis showed certain agents were significantly and repeatedly associated with febrile illnesses. These included adenoviruses types 1, 3, and 5, influenza A, parainfluenza 1 and 3, poliovirus 2, Coxsackie B5, Group A streptococci 4, 12, 23; Shigella sonnei, and, of course, measles and chickenpox viruses. Others, including many respiratory and enteric viruses and bacteria presumed to cause illness, failed to produce sufficient illnesses in excess of the high "normal" rates to provide significant associations. Although these latter observations do not exclude the likelihood that under other circumstances they might well produce clearcut illnesses, repetitive occurrences of viruses and bacteria over several years in this carefully studied population provide valuable information on the relative pathogenicities of many common agents.

DEFINITION OF SPECIFIC VIRAL DISEASES

Perhaps of even more importance at this time are the specific and original contributions to our growing fund of information about the existence and behavior of new human viruses, first discovered or delineated in our Junior Village studies.

Investigators found Junior Village valuable in developing much new information on the natural occurrence of salivary gland virus, particularly its continuous presence in the oral secretions of many infants. They discovered the first hemadsorption viruses (parainfluenza 1 and 3) in Junior Village specimens, and the first outbreak defining the clinical importance of parainfluenza 3 was reported in this population. In 1959 three large outbreaks of parainfluenza 3 showed that although reinfection of infants with serum antibodies occurred, increasing levels of antibody provided increasing protection against both infection and illness. Ten percent of 120 cases had pneumonia - all of them occurred in infants without antibodies or other evidence of previous infection. Recently a medical virologist was able to confirm his discovery of

a fourth parainfluenza virus during an outbreak of infection with this virus in Junior Village. Others, using the Junior Village study system, finally developed conclusive evidence that most of the adenoviruses that commonly infect children also cause significant amounts of illness - nearly 50 percent of the infections with types 1, 3, and 5 showing clearcut evidence of association with febrile illness. Similarly, a number of new serological prototypes of adenoviruses, including a group of them that can be demonstrated only in the intestinal contents were discovered. The natural occurrences of ECHO 10-like viruses observed in this population provided the necessary information employed in 1959 to construct the three separate prototypes, the new reovirus group. The new HI techniques used for diagnosis of infections with these agents were also developed using serums from patients in Junior Village with demonstrated reovirus infections. This procedure was used to determine much of what is known about the epidemiology of these agents.

RESPIRATORY VIRAL VACCINES

In 1959, preliminary controlled tests of commercially produced experimental respiratory virus vaccines were initiated in Junior Village. Adenovirus, influenza, Coxsackie B, parainfluenza and measles vaccines, all of them inactivated by formalin, were placed under study in the orphanage. Recently a multivalent vaccine, including 12 different antigens, was also introduced. Except for suggestive evidence of some protection from measles vaccine, the Junior Village vaccine studies have served chiefly to re-emphasize that antibody response of young infants to inactivated viral antigens is remarkably less than that of adults and that inactivated vaccines of much greater antigenic potency will be required to support any reasonable hopes for producing adequate protection in young children.

SEROEPIDEMIOLOGIC STUDIES

The development and evaluation of suitable methods for acquiring large amounts of data on viral infections in large general and selected populations represents perhaps a major current activity in the field of viral technology. Thus virologists have now developed complement fixation and HI procedures for measuring type specific serum antibodies to more than 40 viruses and with the use of group specific antigens it is now technically possible to test for antibody responses to 75 or 80 viruses.

This represents a major advance which can now be exploited to carry out really comprehensive seroepidemiological studies of human viral experiences in any special or general frame of reference. Thus differences between the prevalence of many agents can now be determined for different times, different geographic areas, different economic or social groups, and different ages. The occurrence of common viral infections of both wild and domestic animals and the likelihood of some of them being shared between species, can now be studied. Well controlled seroepidemiologic studies will be of particular value in developing leads concerning needs for viral vaccines and the roles of viruses in neonatal, chronic, and neoplastic diseases.

The seroepidemiologic studies were much advanced in 1959 through the collaborative study undertaken with NINDB, particularly through a contract with Microbiological Associates, Inc., which has successfully developed and produced in large amounts the majority of viral antigens needed.

SPECIFIC RESPIRATORY DISEASE STUDIES

The role of the "Eaton" agent in Primary Atypical Pneumonia (PAP) has been a controversial one for many years. Furthermore, to those who accepted it as one of the causes of nonbacterial pneumonia the importance of the agent as a cause of disease and its natural history remained unknown. LID scientists with the Bureau of Medicine and Surgery, USN, studied longitudinally continuous endemic occurrences of primary atypical pneumonia at a Marine recruit training center in the South. The Coons fluorescent antibody technique adapted in LID for large scale routine diagnosis of PAP made the study possible. Approximately 70 percent of all pneumonia observed was associated with Eaton virus infection, whereas 23 percent of persons with febrile respiratory illness and 6 percent of persons with no respiratory illness showed rises to PAP. The ratio of illness to infection was approximately one in 12. The existence of this rather large clinically inapparent segment of PAP was unexpected. The data obtained from military recruits was supplemented by information on the occurrence of PAP in children observed in the cooperative study at Children's Hospital. Here it was found over a 16 month period that 10 percent of lower respiratory illnesses was associated with infection with the Eaton agent.

RESPIRATORY VIRUSES POSSIBLY SHARED BETWEEN MAN AND DOMESTIC ANIMALS

The demonstration in 1958 that parainfluenza 3 virus could be isolated from young cattle with respiratory disease and/or shipping fever led to further studies of the epidemiology of this and other viruses in the bovine species. Cooperating with the University of Maryland Division of Veterinary Science, and the University of Illinois School of Veterinary Medicine, parainfluenza 3 was found to be one of the most common virus infections of cattle - often producing little or no clinical disease. As in man, reinfections with the virus were also demonstrated. Despite occasional illnesses produced in calves by artificial inoculation and the recovery of the agent from pneumonic lungs the importance of the virus in producing shipping fever was not determined. Experimental vaccines, with and without adjuvants produced antibody levels capable of modifying the virus propagation on subsequent challenge. However, field studies designed to determine the ability of the vaccine to protect against shipping fever were vitiated by the failure of shipping fever to occur even in controls in the one study that could be completed.

STUDIES OF TUMOR (OR CANCER) VIRUSES

Now a major research effort in the Virus Section, studies of animal tumor and cancer viruses have been aimed at three unsolved problems that appear to be interfering most with maximum progress in tumor virus research.

The first problem is the lack of simple viral techniques for studying most tumor viruses in the laboratory and for recognizing their occurrence in their natural hosts. Although signal advances have been made in the laboratory study on polyoma, Rous sarcoma, avian leukosis and Friend luekemia viruses, most tumor virus activity must still be measured entirely by the production of tumors - a very clumsy, slow and uncertain method.

In 1959, LID scientists applied new viral techniques to studies of pathogenesis, modes of infection, excretion, laboratory spread and natural occurrence to a number of viruses thought to be tumor agents - most notably polyoma virus, Friend leukemia, and the presumed avian lymphomatosis tissue culture agent.

Polyoma virus was shown to grow to extremely high titers in certain organs, 10 to 20 days after infection of the newborn mouse. Hemagglutinins, complement-fixing antigens, inhibitors and specific antibodies in addition to infectivity were compared in a number of different tissues and excretions at regular intervals from three days to three months after infections. Remarkable differences in titers of these various attributes were noted between various tissues. The most sensitive method for recovering virus from polyoma tumor tissues proved to be cultivation of the tumor tissue itself when infection was produced in infancy. Virus could often be demonstrated regularly beyond three months and in some mice appeared to persist throughout life.

Infection of weanlings led to significantly less multiplication of virus and excretion was minimal. Our serological group furnished information to other cancer research laboratories on the prevalence of spontaneous polyoma and extrinsic virus infections in their passage materials and breeding colonies.

The second problem, a very complex one, is produced by the numerous "extrinsic" viruses that sooner or later contaminate laboratory tissue culture study systems and also (not unexpectedly) the experimental animals used for studying tumor viruses. During recent months a number of viruses occurring as "fellow travelers" in many mouse tumor virus study systems were demonstrated. Passage materials and host animals used for passage were often found to contain not only one but multiple extrinsic viruses. Some of these viruses encountered had been recognized previously, such as: K virus, mouse salivary gland virus, LCM, mouse hepatitis. Others were new agents related to human adenoviruses, reoviruses, and even polyoma. The latter virus, perhaps now the best studied tumor virus available, was found in many different laboratories also to be a notorious contaminant of other tumor virus study systems. It has been found in passage material of Gross leukemias, Schwartz leukemia, Bittner mammary cancer, and even human tumors, but its association with the characteristic manifestations of those tumor systems appears to be wholly spurious. Thus tumor viruses are found complicating the study of other tumor viruses, which really is not so surprising in that latent viruses are more likely than "acute" viruses to persist in tumor tissues. Interestingly enough, virtually all the viruses so far found in the tumor study systems appear to be latent agents - and perhaps for this reason themselves are possibly suspect as tumorigenic agents. Recently, we studied the tissue culture grown RPL 12 virus, widely used in model study systems, as an example of visceral lymphomatosis virus - an avian leukosis tumor virus. An extensive seroepidemiologic study using a tissue culture neutralization test showed almost conclusively that the presumed tumor agent being propagated in tissue culture was not the virus which causes avian lymphomatosis but a

biologically similar latent agent, undoubtedly picked up, as would be expected, during numerous passages of lymphomatosis tumor virus through chickens. This tissue culture virus apparently occurs in as many as 90 percent of the breeding animals in chicken colonies in various parts of the country, thus assuring a high rate of association of antibodies to the tissue culture agent with lymphomatous chickens. All chickens involved in several clearcut outbreaks of classical lymphomatosis were negative.

The third critical need is information on the natural history of tumor viruses. Almost without exception contemporary tumor virus studies are confined to artificial laboratory study systems utilizing chiefly special categories of mice, chicken or tissue cultures. These various and quite valuable modes of approach to tumor virus study are meant to be "models" of what (it is hoped) happens in nature. This in part is frustrated by the "extrinsic" viruses. However, until the natural occurrences and biology of these agents are known, at least in part, their real importance as biologic entities and as causes of cancer must remain obscure.

STUDIES OF TUMOR VIRUSES IN NATURE

To answer these and other questions about the natural history of polyoma virus we decided in July, 1959 to do now what had to be done eventually, namely, study the occurrence and behavior of polyoma in Mus musculus, and in their natural environments, both rural and urban. Without exception, Mus musculus and other rodents in rural areas have so far failed to reveal any evidence of infection with polyoma. When the first 500 mice trapped in 43 different areas in lower Manhattan, Queens, Brooklyn and the Bronx showed widespread infections with certain other viruses but absolutely no evidence of polyoma, serious questions were raised about the origins and natural hosts of polyoma. This question was quickly resolved when we turned to systematic surveys of mice in the more densely populated areas of Harlem. Here varying percentages of individual mice in more than half of the mouse populations surveyed were serologically positive. A field laboratory was immediately established in Harlem with the assistance of the New York City Department of Health. The story is quite incomplete - however, the following was determined about the natural history of polyoma.

All newly developed techniques for assaying polyoma (HI, CF, MAP, tissue culture isolation and neutralization techniques) confirmed the widespread presence of polyoma in the extremely dense mouse populations of certain tenement areas in central Harlem. In this area nearly 20 percent of approximately 1000 mice of all ages and sizes showed serological evidence of polyoma; polyoma virus was isolated from tissues and urine of 25 percent of the serologically positive mice, and at least two of the wild strains were shown to be highly tumorigenic.

Although arthropods (A. sanguineous, L. bacoti, and hair mites), and many insects (particularly roaches) were observed, often in great profusion, the studies failed to suggest that they played an important role either as reservoirs or vectors.

Polyoma virus was readily demonstrated in mouse nesting materials. This was consistent with our previous experimental observations that polyoma is excreted in large amounts from newborn mice, but inconsistent with our data on spontaneously infected adult laboratory mice which apparently did not. However, certain naturally infected adult wild mice excreted considerable amounts of polyoma. This information provided the basis for a hypothesis which could account for the natural spread of polyoma. This hypothesis recognizes the fact that certain mice, including adults, become chronic carriers and shedders of polyoma virus, an agent well equipped to survive outside the body. Also postulated were mouse populations large enough and dense enough to furnish a constant supply of susceptible mice, and an environment in which mice shared community nests contaminated with polyoma virus; all circumstances observed in the Harlem tenements. Since data previously reported showed that mice could be infected easily by oral and respiratory exposures to polyoma virus, the hypothesis seems to satisfactorily explain much of the natural spread of polyoma. So that even in nature polyoma virus behaves much like an ordinary highly resistant respiratory or enterovirus. A number of rats and 60 humans living in the apartments containing heavy mouse and environmental infections with polyoma showed no definite evidence of specific polyoma antibodies. A number of mouse viruses were also encountered in the wild mice, including several related to common human viruses; and, as continues to be the case in nearly all laboratories studying tumor viruses these agents created many problems of a technical nature as well as difficulties in interpretating the data.

STUDIES IN MEDICAL MYCOLOGY

NIAID mycologists have continued to find ecologic associations of pathogenic fungi with certain specific types of soil or organic material. Cryptococcus neoformans has been isolated from many additional sites where pigeons roost or nest. Virulent strains of the fungus have been isolated from attics of old open buildings and window ledges outside office buildings. Detailed studies of microflora of Histoplasma positive soil samples taken adjacent to a building housing bats and comparative studies of Histoplasma negative soil samples taken a few feet away have not as yet revealed a recognized difference in fungal flora, except for presence or absence of Histoplasma.

They have continued to test antifungal drugs in a standardized in vivo test using mice. Others have collaborated by making and supplying drugs of one series which was systematically examined and by testing promising drugs in clinical trials. One antibiotic, found three years ago to surpass other antibiotics in experimental therapy studies, has now had encouraging clinical application.

In collaboration with the Bureau of Medicine and Surgery of the Navy, the etiology of a febrile illness occurring in underwater swimmers was investigated at Key West, Florida. Evidence has been obtained which indicates that this may be an allergic manifestation of exposure to a fungus in persons already hypersensitive to this fungus. Several fungi were found growing in the masks used and three of these are still under investigation in experimentally hypersensitized animals and by field use of an antigen prepared from one of them. Experimental studies of two fungi and standardization of antigens prepared from them are incomplete. As a result of these unfinished studies better sanitation of the masks has been enforced and apparently has controlled the febrile disease.

BIOCHEMISTRY OF ANTIBIOTICS

A study is being made of staphylococcal penicillinase both in the whole cell and in cell-free extracts. Emphasis is placed on the uncovering of an inhibitor for staphylococcal penicillinase. Since the penicillin nucleus is composed of cysteine and D-valine, it was thought possible that certain dipeptides could inhibit the activity of the enzyme. This has proved to be the case. Numerous dipeptides have been found to exert an inhibitory effect and, in particular, D-valyl D-valine has been found to inhibit strongly both cell-free and whole cell staphylococcal penicillinase. An offshoot of this work on the inhibition of penicillinase has yielded the surprising observation that relatively simple alcohols strongly stimulate the rate of penicillinase activity. Indeed, stimulations in rate up to 35 fold have been obtained in the presence of n-propanol. As an operational hypothesis, it has been postulated that propanol is active by virtue of the removal of a naturally occurring inhibitor present in both whole cells and cell-free penicillinase.

STREPTOCOCCI AND OTHER HUMAN BACTERIAL PATHOGENS

A relatively new method for the isolation of M protein from Group A streptococcal cells is now used. A streptococcal cell wall enzyme, lysin, is prepared from fresh phage lysates of Group C streptococci and is reacted with Group A streptococcal cell walls. The resulting digest, when fractionated by various means, including ion-exchange column chromatography yields an M protein which compares favorably in respect to antigenicity to the M protein extracted by conventional means. Type specific antibodies as demonstrated by various tests developed in rabbits after intracutaneous injection of 1.5 mg. per animal. Following such

injections, no toxicity was noted. In the course of these studies, it was noted that lysin M as well as purified acid M and crude acid extracts from Group A streptococci contained a factor which precipitated mammalian plasmas and purified fibrinogens. This so-called FPF (fibrinogen precipitating factor) is under investigation to determine its biological significance.

FRACTIONATION OF HYDROGEN ISOTOPES BY A MARINE PSEUDOMONAD

A marine Pseudomonad isolated from the Bahama Banks has been found to enrich with respect to deuterium as compared to light hydrogen to the extent of 20-fold. Similar results have been obtained using tritium. In both cases, the enrichment exceeds by far that which can be expected from exchange reaction equilibria. Warburg studies and biochemical analyses have shown that the initial fermentative degradation of glucose is by the Embden-Meyerhof scheme. Hydrogen arises presumably from glucose via formate. Formate is decomposed to CO_2 and H_2. One and perhaps two separate sites for deuterium discrimination has/have been fairly well delineated. One is the glucose-pyruvate step and the second occurs between formate and CO_2 and H_2. Practical implications of this study include the possibility of devising a cheaper, more efficient fractionation of the hydrogen isotopes.

Q FEVER STUDIES AT ROCKY MOUNTAIN LABORATORY

Possibly the most significant expansion of the research program at the Rocky Mountain Laboratory has resulted from renewed interest in Q fever occasioned by our findings that this disease is present and spreading among dairy cattle in the United States and that a large number of cases of severe illness have occurred in persons residing in Twin Falls County, Idaho. Concomitant with discovery of human cases, serologic surveys of certain population groups in Idaho were performed, revealing the presence of a large number of persons who had specific antibodies against Coxiella burnetii even though it was not possible to elicit evidence of clinical illness. Epidemiologic investigation of persons engaged in the dairy trade in the Twin Falls area revealed that those associated with infected herds had more evidence of illness than those whose herds were free of Q fever. Similar studies are now being done in Ada County, Idaho, an area where only serologic evidence of disease has been encountered.

The situation in Montana can be illustrated best by quoting figures collected by RML investigators. Among 8,000 dairy herds in the State there are 119 containing infected animals. Most infections are concentrated in the milk sheds and only near Kalispell are herds found to be free. In Ravalli County, the herd infection rate is 4.2 percent; in Gallatin County and Teton County the rate is 7.0 percent and 10.0 percent. Among 11,277 human serums examined, 162 or 1.4 percent were found to have antibodies against C. burnetii. While these figures indicate that Q fever is a problem in Montana, data accumulated on the spread of infection in dairy cattle are more enlightening. Dairy cattle from selected dairy sheds were surveyed for the presence of infection in February 1959 and 45 of 1,741 herds were found to be infected. Similar surveys conducted in October 1959 showed 119 of these herds to be infected. In eight months the herd infection rate had increased from 2.6 percent to 6.8 percent.

The discrepancy between the number of clinical human cases, serologically positive persons, and the presence of a vast animal reservoir of infection is of interest. Although only a very few clinical cases have occurred in Montana, it has been found that these persons have been associated with cattle from which it has been possible to isolate organisms of about the same degree of virulence encountered previously in California. The questions of whether or not relatively avirulent strains exist in cattle, whether or not antibodies present in infected milk influence the course of human disease, or whether some other animal such as sheep is a factor in determining human reaction, are being studied. In Ravalli County, studies have been initiated to determine if and how human disease may develop during a period when the disease is spreading among dairy cattle.

Laboratory investigations have been directed largely toward production of C. burnetii on modified Zinsser tissue cultures, purification of these organisms, and extraction of rickettsiae to obtain antigens. C. burnetii can be extracted with dimethyl

a nucleoside, and a component acting as a coenzyme in the [illegible] system has been obtained quantitatively. When C. burnetii is grown in tissue cultures, there is an increase in the number of organisms and in the neighborhood of 1 to 2.5 mg of rickettsiae can be obtained from each gram of tissue. By judicious application of continuous sucrose gradients, it is possible to purify the organisms so that no contaminating material can be observed by electron microscopy. Recent results show glycerol-water gradients to be equally as valuable in this purification process. Since the entire procedure is one which has been adapted to relatively large-scale experimental methods and is one in which chick tissue need not be employed, it is obvious that it has application to many problems dealing with immunity and sensitivity in Q fever. The problem of sensitivity will be dealt with in the very near future.

COLORADO TICK FEVER

One of the very interesting observations coming from field investigations on Colorado tick fever is that the incidence of ticks infected with rickettsiae in areas where many ticks are found to harbor CTF virus is practically nil. Results of preliminary experimental studies indicate that ticks which have been infected as larvae or nymphs with CTF virus prior to or after infection with R. rickettsiae yield only CTF virus when tested for both organisms after they have moulted to the adult stage.

In further studies of the ecology, RML scientists have been able to re-emphasize the fact that microecological conditions greatly influence the incidence of infection of rodents and ticks with CTF virus. In Montana, at least, the presence of golden-mantled ground squirrels greatly facilitates the maintenance and spread of virus. Studies were made of the duration and height of viremia in native rodents, and it was found that viremia in this rodent is both prolonged and of sufficient severity to account for infection of ticks feeding upon them.

Laboratory studies have produced an antigen from brains of mice infected with CTF virus which is effectively killed and still is capable of producing neutralizing antibodies in recipient animals. Present preparations are inactivated at 4° C for periods as long as 60 days with 1:4,000 formaldehyde before all virus is killed. It is interesting to note that with the old vaccines there was evidence in human beings that while the occurrence of disease was not prevented, the clinical illness appeared to be considerably less severe than in nonimmunized persons. That this disease is by no means an uncommon one in the West is demonstrated by isolation of virus from 553 patients to date and by the observation that 8 percent and 3 percent of serums from persons living in rural areas of Wyoming and Idaho possess neutralizing antibodies against CTF virus.

Considerable interest has been manifested in the strain of virus isolated in Powassan, Ontario. A field investigation was most

valuable in de-emphasizing the idea that this virus might be transmitted by ticks, since few ticks are present in the area and the periods during which cases occur do not coincide with periods when ticks are active. Laboratory studies likewise have shown this virus to have, at best, only a remote serologic relation to the viruses of Russian spring-summer encephalitis and of louping ill. It is of interest that serums from residents of rural areas of the West and Alaska were all negative when tested for antibodies against this virus

BAT RABIES

The presence of rabies in bats has continued to be a problem. This year isolations were made from eleven bats, representative of three genera and four species, including Eptesicus fuscus, Myotis lucifugus, M. evotis, and Lasionycteris noctivagans. Three isolations were made directly in mice following bat bites. It is regrettable that the cooperative project contemplated with the University of Montana did not materialize since it is of extreme importance that population studies be instituted in order to obtain valid information on the importance of bats as a potential source of infection for man and animals and to establish bat colonies in the laboratory so that experimental studies are not limited in time. While attempts will be made to continue to do these things, progress will be severely hampered by lack of support of the proposed cooperative program. The presence of Histoplasma capsulatum in bats was noted.

FRACTIONATION OF CELL WALLS

A great deal of interest has been manifested by a number of investigators in the techniques for the physical isolation of cell walls and protoplasmic fractions of various organisms. Isolation of these fractions makes it much simpler to collect and purify antigenic materials useful for diagnostic or immunological purposes. In conjunction with the University of Montana, investigations were made of the role of cell walls and protoplasm of Bordetella pertussis in producing certain effects in mice. It had been postulated previously that cell walls of this organism might confer protective immunity to mice, but no definite proof of this had been obtained. Our experiments showed that protoplasm contained a heat-labile toxin which was toxic both for mice and for the skin of rabbits and that the cell walls contained the histamine-sensitizing factor and the protective antigen.

In conjunction with a scientist from the University of Georgia, studies were made of the components of Brucella abortus to determine their toxicity and their potentialities as protective antigens. Cell wall and protoplasmic fractions were obtained and a water-soluble material was obtained by the extraction of whole cells with ether. When studied in mice, it was found that the protective index conferred by these various materials was as follows: controls = 0, protoplasm = 1.0, acetone-dried whole cells = 1.9, residue ether extract = 2.7, cell walls = 3.7, and aqueous ether extract = >4.6. The cell walls and the aqueous ether extract are potent endotoxins for mice. The antigen extracted from Br. abortus by ether is soluble in water and is toxic. It resembles similar antigens obtained from Salmonella in these respects, but differs from that of Bacterium tularense since

in the latter organism this treatment results in the isolation of a particulate nontoxic fraction.

Thus, the studies carried out this year serve to extend observations made in the past which emphasize the importance of cell wall fractions and of the cell wall itself in producing protective immunity in experimental animals. This upholds the theory that protective antigens of bacteria should be located upon the surface of the organisms rather than in the interior. Studies with Salmonella have been directed mainly toward determining the effect of lipid which is contained in the so-called complete antigens. According to Westphal, the lipid contained in this protein-carbohydrate-lipid complex is both toxic and immunogenic. This idea is difficult to accept in view of the evidence accumulated that carbohydrates and proteins are in general responsible for immunogenic reactions.

Studies carried out at the Rocky Mountain Laboratory and in conjunction with the National Cancer Institute, show that the lipid fraction of complete antigens is not responsible for many of the biological activities of complete antigens of Salmonella. By reducing the lipid content of antigens from approximately 19 percent to one or two percent and testing the original and the delipified antigen by various methods, it was found that the delipified material reacted, by and large, in the same way as the normal antigen. Thus, they were toxic, they produced pyrogenic reactions in rabbits, they produced skin lesions in rabbits, they had a tumor-damaging factor, they were toxic for thorotrast-treated rabbits, and both conferred specific and non-specific protection upon mice which were immunized with them. It was found that lipid did not produce specific protection in mice, but was capable of inducing non-specific resistance.

ANTIGENS FROM TUBERCLE BACILLUS

The original enthusiasm for concentrating efforts to obtain cell wall fractions of tubercle bacilli to immunize animals against tuberculous infection has been dampened considerably by the consistently negative results obtained in immunized mice challenged by the pulmonary route. While it has been possible to show that killed antigens are capable of protecting mice against infections produced by intravenous inoculation of virulent organisms, it has not been possible to demonstrate that cell walls, protoplasm, or phenol-killed antigens are capable of producing significant immunity in mice which are subsequently challenged with a relatively small dose of virulent organisms given by aerosol. On the other hand, preparations of living BCG or H37Ra will effectively immunize mice against aerosol infection, and the dose of avirulent organisms given as vaccine may be considerably diluted and still confer protective immunity. It has also been demonstrated that significant immunity persists for at least 26 weeks after administration of vaccine. One of the interesting aspects of this study has been that the results in mice are similar to those obtained in human populations where it is stated that the incidence of pulmonary tuberculosis is not greatly affected by vaccination with BCG, but the incidence of tuberculous meningitis and miliary tuberculosis and other secondary

granulomas is greatly decreased. In our experimental system it can be shown that bacteremia is uncommon following infection of immunized mice, whereas organisms are found consistently in the lungs even in the absence of tubercles. As a basis for a potency test for living vaccines to be used in man, this system appears to be quantitative and to result in a graded immunity response in mice.

With cell walls obtained from Mycobacterium butyricum and M. tuberculosis, it has been possible to demonstrate the specificity of the hypersensitivity produced in rabbits by injection of one or the other of these fractions. There is considerable need for this type of test and, in conjunction with serological studies, it may be possible to demonstrate chemical and antigenic differences in various species and strains of mycobacteria.

STUDIES ON DELAYED HYPERSENSITIVITY

Continued investigations serve to add emphasis to the theory that delayed hypersensitivity is a preliminary and essential step in production of immediate sensitivity and circulating antibodies. This relation of delayed to immediate hypersensitivity was strengthened by demonstration of the anamnestic response displayed by animals receiving a primary dose of antigen so small that they develop only delayed hypersensitivity but develop circulating antibodies very rapidly if a secondary stimulus is applied at the time delayed hypersensitivity is at its height. Since delayed hypersensitivity to a protein-haptene complex is directed toward the broad protein portion of the moiety and immediate hypersensitivity toward the specific haptene portion, it is evident that specificity of the immune reaction in animals changes as the process matures. These data showing that during the process of developing immunity there is a broad area toward which antigens are directed suggest that administration of one type of poliovirus (type I) may, very early, produce immunity to heterologous types (II and III) which may be accelerated and increased by secondary injections of the heterologous types of poliovirus.

Antigens of Trichophyton rubrum are being employed to study the role of hypersensitivity in skin infections due to this organism or to T. mentagrophytes. Patients suffering from infections with the latter organism display delayed reactions to the antigen, but patients infected with T. rubrum fail to do so, suggesting that the reason for the development of chronic infections with T. rubrum is due to its lack of antigens which are responsible for production of immunity.

PURIFICATION OF VIRUSES

From a practical point of view, the discovery of methods whereby large quantities of infected tissue-culture fluid can be concentrated and virus purified makes it feasible to produce purified virus for immunogenic studies. Routine harvests of 5 X 10^{11} PFU of virus are obtained. Based on 10^{6} PFU as a suitable immunizing dose for man, about 500,000 doses of vaccine are produced with limited experimental facilities. From a theoretical viewpoint,

data indicating that the RNA of poliovirus is made up of a single strand which can be inactivated by disintegration of a single bond are fundamental to studies of proliferation of animal viruses. Stress is placed upon observations of the failure of S^{35} and P^{32} of virus used to infect cells to be incorporated in significant amounts into new virus. These observations differ from those made regarding tobacco mosaic virus and bacteriophage. It should be noted that labeling of poliovirus with S^{35} contained in methionine serves to mark the protein fraction of the virus in contrast to the marking of nucleic acid which is accomplished with P^{32}. Labeling of protein of poliovirus by this method has not been reported previously.

TICK PARALYSIS

Studies of tick paralysis have continued and established that hamsters, marmots, dogs, foxes, and monkeys develop disease when female ticks are allowed to feed upon them. An interesting observation which may have some relation to the transmission of tick paralysis is the appearence of a previously undescribed fat-like body developing in female ticks during engorgement. In conjunction with scientists at the University of Utah, investigations of the pharmacological action of the toxin have been expanded, and it is now definite that the central as well as the peripheral nervous system is involved in this disease.

GERMFREE ANIMAL RESEARCH

Significant program development occurred along two major lines during the year. First, the germfree mouse colony, initiated at the end of last year, increased to the point where these animals became available for experimental use. In the past, the Institute's research program in this field had been limited to the use of germfree guinea pigs. Secondly, and undoubtedly associated with the first, there has been an increase in the variety and number of cooperative projects either planned or underway with other Laboratories.

A study of the lesions produced by Entamoeba histolytica associated with a variety of individual species of bacteria, has shown that only mild to moderate infections are usually produced by some species, e.g., Escherichia coli and Streptococcus faecalis. On the other hand, a more acute ulcerative amoebiasis occurs when either Bacillus subtilis or Lactobacillus acidophilus is present. These findings may explain, in part, the varied picture that infection with this parasite can present.

Germfree animals, especially guinea pigs, have notoriously enlarged ceca. On the "natural product" type of diet ordinarily used to raise this species germfree, this organ and its contents can comprise up to 40 percent of the animal's body weight. With the use of semisynthetic diets sterilized by irradiation, a scientist has obtained germfree animals with an average cecal weight of less than 10 percent of the body weight, a figure which approaches that of the conventional animal. These studies may point out ways in which the lack of an intestinal flora affects the physiology of histology of the intestine.

In another type of study, we had shown earlier that the protozoan parasite, Trichomonas vaginalis, multiplied virorously and produced large lesions when inoculated into the loose subcutaneous tissues on the back of germfree guinea pigs. However, for the most part, the organisms disappeared in conventional animals. Usually, a tiny fibrous noduel was all that remained, if anything, two weeks after inoculation. Recently, we have observed that if the germfree animals are orally contaminated with a single species of nonpathogenic, slowly-growing Micrococcus isolated from conventional guinea pig feces, and then are inoculated two weeks later, results resemble those obtained in the conventional animals. The presence of even a single "mild" species of bacterium appeared to stimulate seemingly nonspecific defensive mechanisms that were lacking or inadequate in the germfree animal. This animal-parasite system may provide a good device for the study of the role ordinary contamination may play in maintaining "natural" defense mechanisms.

As an example of the extra-Institute collaboration we attempt on a limited scale, we have just completed a study in which it was demonstrated that bacteria are not essential for the development of periodontal disease in mice. This appears to be true, also, for the development of calculus on the teeth. It is to be recalled that bacteria were required to produce dental caries in the rat.

In collaboration with the Laboratory of Tropical Virology, we have initiated studies on the behavior of viruses in the germfree host. It was noted that germfree mice survived somewhat better than did conventional mice following inoculation with either dengue type 2 virus or Japanese encephalitis virus. The differences, though not great, were significant. This finding would be of special biological interest if noted with other viruses, particularly in view of the fact that germfree animals have tended to be more susceptible than the conventional ones when challenged with other types of organisms.

GERMFREE MOUSE KIDNEY TISSUE CULTURE

One of the most intriguing things noted in this collaborative project has come from the use of germfree mouse kidney tissue culture. It was thought that tissue cultures from cells of animals which, for many generations, had not had to contend with a variety of infectious agents might possibly provide an especially favorable growth medium for viruses. If true, this might be more easily demonstrable with viruses that do poorly in conventional mouse cell lines. In parallel tests with dengue type 1 virus (the Mochizuki strain), marked differences in virus multiplication and production of cytopathogenic effects were noted between the germfree mouse kidney tissue culture and those prepared from mice of the NIH colony. In the latter CPE was usually equivocal and titers ranged from 2.5 to 4.5 log $TCID_{50}$. In the germfree animal cell cultures, CPE was marked and titers as high as 7.5 log $TCID_{50}$ have been obtained. We are now using mouse kidney cells from the LGAR conventional colony, derived originally from the germfree mouse colony, to assess the possibility that a genetic or strain difference is involved. In our first simultaneous trial of cells from the three groups of mice, both conventional lines gave about the same result, but viral growth and CPE were much greater in the germfree mouse kidney. Thus, it would appear that the latter provides a good system for detecting dengue (M) virus.

As yet, of course, we do not know whether or not this is a general phenomenon or merely peculiar to this strain of virus. It is likely, however, that additional viral agents will be tried on germfree animal cells. If such cultures prove, in general, to be an especially sensitive system, their use for the detection of viral agents in suspect clinical material may develop. In anticipation of this we plan to increase our supply of germfree mice.

MIDDLE AMERICA RESEARCH UNIT

During the year the Virus Section of MARU has grown from a field station into a research laboratory engaged in investigations which range from emergency workup of influenza epidemics to controlled laboratory studies of pathogenesis and experimental transmission of encephalomyocarditis virus in swine; as planned the primary emphasis has nevertheless remained on the arthropod-borne virus diseases. The staff has applied, modified or developed techniques utilizing most of the diagnostic armamentarium of a modern virus research laboratory, including extensive use of several tissue culture systems, animal and egg inoculation, immune serum production and serological techniques of neutralization, hemagglutination-inhibition, hemadsorption-neutralization and inhibition, and complement fixation. To our knowledge the range of methods and techniques employed is not duplicated in any other laboratory facility in the Americas, outside of the United States and Canada.

Probably the most important activity now in full progress is the full scale inquiry into the ecology of arthropod-borne viruses in Panama conducted in collaboration with the Gorgas Memorial Laboratory. Collections of mosquitoes and phlebotomus flies have been carried on daily since November 1959. Of the approximately 200 arthropod pools processed and routinely inoculated into suckling mice (inoculations performed in duplicate at MARU and GML) and into hamster kidney tissue culture tubes, several isolates have been made. Most significantly, two isolates were made from phlebotomus pools, one of them of a virus quite unfamiliar in its biological characteristics. The 18 months' study of the eastern equine encephalomyelitis virus in Panama resulted in elucidation of the role of this virus infection in horses and human beings in Panama and disclosed the presence of antibodies evidencing infection not only in certain birds and mammals, but also in reptiles. The encephalomyocarditis virus has been found to be the cause of a fatal disease in swine; its possible pathogenesis and transmission are being investigated. This serves as the first conclusive demonstration of this virus in Central America; the finding may lead to the establishment of a new important zoonosis. Interesting results are being accumulated by examination of the bi-weekly collections of rectal swabs from Panamanian infants under three years of age. This longitutional study has disclosed a periodic fluctuation of certain enteroviruses related and unrelated to community illness. Epidemic influenza outbreaks in Panama (Type B), in British Guiana (Type A_2) and in Costa Rica (Both A_2 & B) were investigated and laboratory support of the Costa Rica national program of live polio vaccine administration was provided.

HISTOPLASMOSIS IN PANAMA

During the year approximately 50 cases of clinical histoplasmosis were confirmed by serological techniques and two fatal cases of disseminated histoplasmosis were confirmed by recovery of the organism from clinical materials (these were in native Panamanians and represented the second reported fatality in a child and the first in an adult). Epidemological investigations of all confirmed clinical cases of histoplasmosis resulted in the first isolation of H. capsulatum from Panamanian soil around a dwelling in which two consecutive occupants developed clinical disease. The ecology and microecology of H. capsulatum remains unsolved; however, the organism has been repeatedly isolated from fresh soil during both dry and wet seasons; naturally occuring histoplasmosis has been demonstrated in spiney rats and opposums by isolation of the organism from liver and spleen. These infections were accompanied by diagnostic serum complement fixation titers. The approaching completion of the histoplasmin and tuberculin skin testing program in the Canal Zone school system will furnish data on approximately 8,000 children between the ages of six and nineteen years. The current preschool age program will supply data on the younger age groups.

ARTHROPOD BORNE VIRUS STUDIES

Certain significant advances in laboratory methods merit individual mention: 1) the development of a practical and specific flocculation test for the demonstration of arthropod-borne viruses and antibodies; 2) demonstration of significant differences in rate of reaction of viruses with homologous and heterologous antibodies which has resulted in practical application of the phenomenon to the heretofore uninterpretable tests of group reactive sera against various ARBOR viruses; 3) evaluation of several available tissue culture systems as practical tools for propagation, quantitation and isolation of certain arthropod-borne viruses; 4) a newly observed phenomenon of "hemonuclear-adsorption reaction" which may be useful as an index of the presence of virus in infected tissue culture systems; 5) studies on the pathogenesis of Ilheus virus infection in adult and suckling mice and of its transmission using mice as hosts and Aeges aegypti mosquitoes as vectors.

BACTERIAL DISEASES

The staphylococcal studies have been directed almost entirely toward development and standardization of tests for use in determination of relative pathogenicity of staphylococcal strains since such base line measurements are essential to further basic studies on the several factors determining pathogenicity of a particular strain. With such a complex organism no single test will serve to characterize the potentials of a strain. It appears that tests determining minimal killing dose (for the mouse), minimal infective dose, and toxic activity, employed in conjunction may serve this purpose. While both bovine and human brucellosis in the United States are decreasing, world-wide brucellosis is one of the major infectious diseases with tremendous impact on agricultural economics as well as a significant cause of human illness. We continue to collaborate with other brucellosis centers in problems relative to diagnostic tests and taxonomy.

MALARIA - HUMAN

This country's commitment to the world-wide program of malaria eradication and the long-term interest in malaria contributed to a research effort, during the past year, largely directed toward problems identified with that disease. Special emphasis was given to malaria chemotherapy as applied to eradication. Inasmuch as regular facilities provide for only a minimum of clinical material, which continues to become less each year, facilities at the Federal Penitentiary at Atlanta, Georgia were again made available. Studies have been in progress there since April, and resulted in more specific knowledge of the action of drugs in man. For instance, single doses of chloroquine (Aralen) as low as 75 mg. (base) per week will result in complete suppression of Chesson strain vivax malaria even under conditions of repeated infection via mosquito bite.

Primaquine given as a single 30 mg. dose, weekly, or as a single 3.0 mg. dose, daily, beginning the day before infection by mosquito bite, did not prevent or suppress infection. The drug is sporontocidal at a dosage as low as 1.5 mg., single dose, daily, against either Plasmodium vivax or P. falciparum.

A strain of P. vivax obtained from the State of Merida in eastern Venezuela, where the population has been under mass suppression with pyrimethamine, was found to have a prepatent period of 13 to 17 days; the incubation period was also 13 to 17 days (data from 10 patients). The parasites of this strain were found to be resistant to a dose of 50 mg. and of 100 mg. of pyrimethamine. This was true whether the infection was induced by blood inoculation or by sporozoites.

MALARIA -IN MONKEYS

Morphological studies of the exoerythrocytic forms in the monkey liver of two strains of P. cynomolgi have been made which will be useful as guidelines in chemotherapy studies and in studies on in vitro growth. Growth has been seen from the fourth through the ninth day and mature parasites have been observed as late as the 120th day. Reaction of the host was lacking in most monkeys, but was found in one animal. Size changes through the cycle have been studied, and it was found that on a given day the size was consistent. Distribution of parasites was found to be uniform throughout the liver and fit the Poisson distribution well.

Cell lines have been established from monkey liver and other sources. Considerable success has been achieved in maintaining organized liver tissue in vitro. Good survival of organized fragments of adult monkey tissue has been obtained for as long as 10 days which is sufficient time for P. cynomolgi and other malaria parasites to complete an exoerythrocytic cycle. The ability to maintain liver is a major step and is basic to studies of the parasites in vitro.

Exoerythrocytic stages of P. cynomolgi have been grown in vitro for two and four days from fragments of tissue taken from heavily infected monkeys. The parasite increased greatly in size during the period of in vitro maintenance. The cytology of the parasites compares well with normal in vivo parasites. This may represent the first in vitro cultivation of mammalian exoerythrocytic parasites.

Monkeys treated with pyrimethamine prior to infection did not develop detectable exoerythrocytic parasites in the liver. Since parasitemia occurred eventually, in some of the monkeys, parasites must have survived at sub-patent levels, survived in a form difficult to detect, or survived in tissues other than the liver.

When pyrimethamine treatment was initiated on the sixth day after injection of sporozoites, an effect was evident within 24 hours and marked after 48 hours. Subsequent to 48 hours, the parasites became vacuolated and disorganized. They showed signs of swelling and by 120 hours after treatment were so altered as to be hardly distinguishable. Very little cellular reaction developed, although occasionally mononuclear cells accumulated around the dying parasites.

When primaquine was given prior to infection, no parasites could be found in the livers. When primaquine was initiated on the sixth day of treatment, an effect was not discernable for 72 to 96 hours after treatment. At 120 hours, after treatment, many parasites appeared as dead, amorphous, dark-staining, shrunken masses without cellular reaction. Studies are now in progress to follow the 'dying' parasites for longer periods.

Both primaquine and pyrimethamine retarded, then stopped the growth of the parasites. This resulted in keeping parasites in the liver at a time the majority would have ruptured and initiated blood infections.

DRUGS - MECHANISM OF ACTION

The results using P. gallinaceum indicate that the drugs fall into groups on the basis of their mode of action. Thus, pyrimethamine and chloroguanide specifically inhibit DNA synthesis, the former under all conditions studied, and the latter only in vivo. The drug effect is reversible only by thymidine, indicating that the drug inhibits the enzymatic conversion of deoxyuridine to thymidine.

Drugs with a quinoline or acridine nucleus (quinine, chloroquine, quinacrine) inhibit respiration, RNA, and DNA metabolism apparently nonspecifically through interference with energy conservation by the parasite. Studies with quinine in greater detail revealed that the drug action is dependent on a high potassium ion concentration surrounding the parasite, such as occurs inside the red cell. Thus, the action of quinine and related drugs on the malarial parasite and heart muscle may be similarly connected with potassium ion metabolism or transport.

In studies involving Pseudomonas sp., pyrimethamine inhibited growth, glucose utilization, and pyruvate utilization to approximately the same extent. At the levels tested, formate utilization was not inhibited. Of many substances tested, pyruvate itself was the only material capable of reversing pyrimethamine inhibition.

Preliminary experiments have shown that in the case of P. gallinaceum, the DNA content of infected cells appears to be no different from that of uninfected cells. On the other hand, the RNA content of infected red cells is five times that of uninfected chicken erythrocytes.

DRUGS - PHARMACOLOGY AND METABOLISM (FATE)

An in vitro system for chloroquine metabolism was found in rabbit liver homogenates. Chloroquine was metabolized at a rate of about 45 percent per hour per gram of tissue at pH 7.4 in an atmosphere of 95 percent oxygen. In the presence of SKF 525-A, Lilly 18947, and pyrimethamine at 1 x 10^{-3} M, chloroquine metabolism was inhibited 80, 90, and 100 percent, respectively. The metabolic activity of liver homogenate was virtually lost after dialysis but could be restored by the addition of a TPN-H generating system. One of the metabolic products is acetaldehyde and evidence suggests that both alkyl groups of the parent compound are oxidized in vitro.

Administration of metabolic inhibitors to rabbits in combination with chloroquine gave rise to higher blood levels and a prolongation of the half-life of the antimalarial drug. However, in most cases, metabolic inhibition beyond 48 hours could not be achieved because of the rapid turnover of the prolonging agent.

DRUGS - TRIALS AGAINST INTESTINAL PARASITES

A total of over 300 cases of hookworm have been treated with bephenium (four different salts) employing a number of dosages and regimens. The more effective regimens, using the granular hydroxynaphthoate salt (Alcopara (R)) at 5 grams base per day, have produced worm reductions of 74 percent (5 grams total base), 93 percent (15 grams base), and 98 percent (25 grams base). The drug is almost completely curative against Ascaris lumbricoides, and higher dosages appear also to have some effect against Trichuris trichiura. Trials of the newer drug dithiazanine iodide (Delvex(R)) against T. trichiura and other helminths continue to confirm the effectiveness of this drug; results against other helminths (hookworm and Ascaris) have not been encouraging. Use of this drug, alone and in combination with the anti-hookworm drug tetrachlorethylene, in the long term mass treatment of the population of an entire building has been carried out. The results are as yet incomplete. After the first 10 months of use of regularly repeated treatments with small doses, it appears likely that elimination of existing worm infections or elimination of transmission has not been achieved.

DRUGS AND NUTRITION AS RELATED TO SCHISTOSOMIASIS

Oral and parenteral regimens of 165 compounds were given to infected mice. Several compounds proved to be weakly schistosomacidal at toxic levels. Two of the compounds were more promising and were studied intensively. The

phenyl-piperazine (Hoechst S 686) previously found to be curative in mice and monkeys was found to have some prophylactic effect in dogs. An oral dose of the compound given on each of the three days following exposure prevented some of the S. japonicum from developing in dogs but this prophylactic effect was not observed in monkeys exposed to S. mansoni. The therapeutic index of S 688 was low in monkeys and dogs.

The second compound selected for trial was an antibiotic, S 1629. A regimen of several oral doses prevented one-half to three-fourths of the worms from developing in mice. In a prophylactic test, no eggs have been found in the feces of a treated monkey while they have been found in the non-medicated control.

NUTRITION IN HELMINTHIC INFECTIONS IN MICE — The importance of nutrition in relation to the cure and chemotherapy of helminthic infections has been shown in studies on mice infected with schistosomes and various nematodes and cestodes. Nicotinic acid deficiency enhances the susceptibility of mice to Schistosoma mansoni infections, but the worms are stunted and incapable of normal egg-production. Folic acid deficiency, with or without B_{12} deficiency, does not alter susceptibility of the host, although infection aggravates the resultant anemia, but decreases egg-production of the worms. Mice on a semi-synthetic diet alone or supplemented with vitamin B_{12} apparently completely lost their oxyurid and tapeworm infections, or harbored stunted Nematospiroides dubius, as compared to controls on a Purina pellet diet. Of great significance is the finding that treatment with stibophen produced much higher cure rates of S. mansoni infections in mice on an enriched semi-synthetic diet as compared to mice on a commercial diet. It is because of these very promising leads that we have initiated the Puerto Rican study on human beings infected with S. mansoni.

The importance of liver disease in parasitic infections has led to a series of studies of general medical interest on the mechanism of ammonia toxicity (12-M). It has been demonstrated that hypoxia markedly increases the toxicity of ammonia in mice. A large number of other factors have been studied but have had only slight effect.

MOLLUSCICIDES — The development of a method for testing molluscicides against snail eggs rather than adult snails is an important technical improvement in this field. Also, the finding that ultraviolet light rapidly inactivates sodium pentachlorophenate is of great importance in explaining failures in the effectiveness of this compound under certain field conditions. Since NaPCP has been demonstrated to be about 10X as effective against snail eggs compared to adult snails, this suggests that low concentrations of the chemical may be useful in maintaining bodies of water snail-free.

METABOLIC CHANGES IN PARASITIC INFECTIONS — Studies on the pathologic physiology of various protozoan infections, as revealed by histochemical techniques, have revealed that infections with Trypanosoma congolense, T. equinum, T. equiperdum, and Bartonella muris show individual peculiarities with respect to liver glycogen and lipid, and that such nonspecific factors as anoxia and adrenal involvement cannot be the only pathogenetic mechanisms. They indicate the possibility that the parasites produce specific substances that influence the observed changes. This has an important bearing in relation to research on chemotherapy and immunology.

The latest studies on the [illegible] which developed from earlier work on the effect of sodium p- chlorophenate on snails have been continued with the demonstration that isolated mitochondria have an appreciable endogenous respiration in the absence of added substrate, and that this is linked with their stability. Only certain reactions of isolated mitochondria have been shown to be affected by the age of the animal; the oxidation of beta-hydroxy-butyrate is impaired with age, but not the subsequent oxidation of acetoacetate. These studies now have a parasitergic orientation as well; it has been shown that the mitochondria isolated from rats parasitized with N. equinum are deficient in oxidative phosphorylation.

TOXOPLASMOSIS — The work on toxoplasmosis has shifted to emphasis on the chronic stage of infection and on the cyst. The nature of the cyst wall, which is of considerable importance in relation to exacerbations during chronicity, has been shown by the electron microscope to be a complicated structure rather than a simple membrane. The occurrence of congenital transmission during chronicity has occurred most frequently with parasite strains that produce abundant cysts and also late parasitemias. Such parasitemias are seen in mice, rabbits, and guinea pigs, but not in rats; in the rat, corticosone occasionally induces parasitemia. The injection of hyperimmune serum into chronically infected mice results in some increase in the number showing parasitemia. This may be of clinical importance in relation to the effect of re-exposures to the parasite on already existing chronic infections. Additional progress in evaluating the usefulness of the hemagglutination test in various animals has shown that the sera of monkeys, pigeons and sheep give good results as compared with the dye test, while sera of rats and dogs do not. Of various fractions prepared from the hemagglutination antigen, one appears to be stable on storage.

AXENIC CULTIVATION — The axenic cultivation of Entamoeba terrapinae for the first time, and of E. invadens, in an improved medium without particulates, represents an important step towards the goal of cultivating E. histolytica without associated microorganisms. Some work on E. histolytica has indicated that the mammalian embryo extracts useful for E. terrapinae and E. invadens may be of value as substitutes for living cells. The usefulness of fluorochrome stains for identifying DNA and RNA in protozoan cells has also been employed in identifying DNA in the endosome of amebae and differentially staining the parabasal body of a trichomonad. One of the most promising developments in this project is the development of cryogenic techniques for storing parasitic protozoa. This will eventually reduce the burden of strain maintenance and also preserve the characteristics of strains.

[illegible] of the Laboratory of Immunology
[illegible] was approved on January 2, 1957, one day
fiscal existence of the Laboratory began
on April 5, 1957.

It seems appropriate at this time to look back and take a view of the activities of the Laboratory of Immunology since this may disclose its aims and policies as well as how close it came to fulfilling them. Although the Laboratory of Immunology is one of the newest units of the NIH, its activities represent the oldest and one of the most significant basic and applied subjects in which the NIH has been engaged. Mention may be made of the international standardization of biologics that received many theoretical and practical contributions from the NIH. The basic studies of many aspects of experimental anaphylaxis -- conducted half a century ago by Rosenau and Anderson -- still exert influence on our thinking on problems of anaphylaxis and certain aspects of allergy now recognized during their scientific epoch.

In starting to organize a laboratory, obviously the main directive is the aim of the laboratory as expressed in its title but this is broad and has many aspects.

The non-clinical part of the laboratory of Immunology started its activity in April 1957. Our numerous attempts to initiate a section of clinical immunology until lately were unsuccessful. However, it now appears probable that in July 1960 this section will begin to operate.

The selection of the personnel (men professional) was guided by the nature of the problem -- availability of space, salaries, and facilities. It was planned to include in the staff workers with experience, who had proved their ability in immunology or in allied fields, and also promising beginners. The latter were included with the hope that they would benefit by working on problems suggested to them or on their own problems. It was hoped that these investigators would soon reach some independence, meanwhile contact with experienced investigators would benefit them. Other workers depending on their interests and experience would work on their problems alone or in small groups. One of the reasons why the idea was favored that the staff should work in small groups or by themselves was that, in general, it is believed that persons with independent minds would always be satisfied to be part of a research group. The situation is strikingly different, of course, when the members of the groups represent different disciplines.

*Report prepared by Dr. Julius Freund, Chief

Most of the problems presented here have been under investigation or will be undertaken in the near future. Some of the work began some years ago. Progress, as in any research program, has revealed new problems or subjects which are being attacked by a variety of new methods. The most important of these is the introduction of genetically distinct, highly inbred guinea pigs into immunologic research work. The initiation of such studies is of particular importance in the field of delayed type of hypersensitivity. The guinea pig is unique in its suitability for the study of delayed hypersensitiveness. In the guinea pig high degrees of local (skin, cornea, scrotal) and systemic reactions (anaphylaxis -- protracted type and delayed type, i.e., tuberculin type) can be produced. In the other experimental animals these types of reactions may occur only in low degree or not at all. Furthermore, some of these reactions may be difficult to distinguish. For example, the delayed type of skin reaction may be exquisitely intense in the guinea pig and mild in the rabbit and hardly demonstrable in the mouse and in the rat using the same preparation, injections.

It appears now that without the use of highly inbred animals certain problems of transplantable tumors could not be profitably studied. Similarly, the use of two highly inbred families of guinea pigs, No. 13 and No. 2, were demonstrated to be essential for homotransplantation and isotransplantation studies of the skin. The transplantation studies of the skin are only of very many immunologic subjects to which inbred guinea pigs may be applied. It is very likely that transfer of many kinds of cell-born passive sensitization can be achieved using inbred guinea pigs. The Chief of the Laboratory suggested experiments to determine whether isotransplantation of the skin within the inbred families and crossing in another breed would yield the results one would expect. These families have been inbred for about fifty years. The Chief of the Laboratory is hopeful to have the gross morphologic specimens of tuberculous guinea pigs of families No. 2 and No. 13 which he examined in 1930 at the Henry Phipps Institute of the University of Pennsylvania. These guinea pigs were infected with virulent bovine tubercle bacilli. When they were killed or died of tuberculosis the lesions were found to be very similar in the guinea pigs of the same families while very dissimilar in the animals of family 13 to the lesions found in family 2. There was an interesting relationship found in these guinea pigs with regard to resistance to tuberculosis and antibody formation. Lewis and Loomis observed that tuberculous guinea pigs of family 13 produced antibodies more abundantly to sheep red cells and killed typhoid bacilli than those of family 2.

The experiments on the transplantation of mouse skin with the aid of inbred families has been successful. Other problems on the transfer of hypersensitiveness to humans are being investigated.

TREATMENT OF FUNGUS INFECTIONS

With the increasing interest in infections due to fungi, it is noteworthy that important advances in treatment are being made by the clinical group. The number of patients (40) at the Clinical Center now treated with amphotericin permits the conclusions that this drug is the first effective treatment for some cases of cryptococcal meningitis and is a superior therapeutic agent in blastomycosis and histoplasmosis. A still newer antibiotic drug, known only by code number, under exclusive study at the NIH has been demonstrated in the laboratory to be even more effective than amphotericin in experimental infections in mice due to Histoplasma and Blastomyces. The use of this drug in 12 patients with a number of infections suggests that it may be superior in human infections due to these same fungi.

CLINICAL STUDIES OF VIRAL DISEASES

In studies of viral diseases, our interest has more and more focused on possible involvement of the kidneys in infection. In addition to clinical and laboratory studies, appropriate to the diseases (respiratory, aseptic meningitis, etc.), special efforts have been devoted to search for virus in urine. In the past two years, six enteroviruses have been found in urine of patients and five of these urinary isolates have been studied in animals. Viral content of mouse kidney at certain times after inoculation is of sufficient titer ($10^{2.5}$ to $10^{4.0}$/mg.) to be poorly, if at all, explained by possible concurrent viremia. On the other hand, recent herpes and vaccinia isolates from man have not been found in rabbit kidney following intravenous inoculation. Virologic studies of urine are similarly being performed to evaluate the significance of urinary cells containing inclusion bodies. Studies of 57 hospitalized patients with ECHO type 9 seen during the summer of 1958 have correlated virologic and clinical aspects of this disease.

STAPHYLOCOCCAL PROBLEM

One aspect of the alarming staphylococcal problem is under unique study here. Investigation into the production and inhibition of penicillinase have revealed that a defined media containing relatively few amino acids promotes the production of this enzyme -- encountered invariably in resistant staphylococci isolated from man. Production of the enzyme has been shown to be independent of bacterial multiplication and to be inhibited by the use of analogues of some of these essential amino acids. Studies of another important bacterial infection in man, Salmonella, have centered around the carrier state, which complication continues to be a problem despite otherwise effective antibiotic treatment. Stones recovered at cholecystectomy in 11 patients have been found infected in every instance, whereas gallbladder wall, liver, and bile were less frequently culturally positive. As a result of these studies,

five stones from three carriers were implanted into the gall-bladders of five rabbits. Salmonella was subsequently and repeatedly isolated from stools for periods now exceeding seven, six, six, four, and two months.

ANTIBODIES IN ASCITIC FLUID

Extension of the technique devised in this Laboratory for the production of potent antibodies in ascitic fluid of mice have shown difference in response of some strains of inbred mice. In addition, plasma cell tumors have been induced by this method in three BALB/CAnN mice. This method has proved suitable for the production of viral antibodies at a great saving in time and number of animals necessary. Continuing studies of patients with lupus erythematosus and agammaglobulinemia have revealed that with a much more sensitive technique of measurement patients with the latter disease do have detectable antibody to a number of enteroviruses. This provides a partial answer to the intriguing observation that patients with this disease seem to handle most viral illnesses well and without recurrence--in contrast to their experience with bacterial disease.

FILARIASIS

Parasitic disease research this past year has been most productive in the problem of filariasis. Transmission of the disease has been shown to occur at low levels of microfilarial density (e.g., 1 microfilaria/20 mm^3 of blood). Mansonella ozzardi infections have been shown to respond less well than Wuchereria bancrofti to diethylcarbaniazine treatment, and tetracycline has been shown to concentrate in the adult Loa loa worm as well as in Dirofilaria immitis and Ascaris lumbricoides. The avidity of filarial tissue for tetracycline is quite remarkable to observe. The phenomenon is distinguishable in vivo, which would suggest a method of diagnosis of the disease. Further, by chemically combining tetracycline with an active anti-parasite compound effective therapy might be achieved.

LUPUS ERYTHEMATOSUS

In association with the Laboratory of Immunology, members of the staff of LCDC have devised and evaluated a test for systemic lupus erythematosus. It is based on a reaction of lupus serum with purified animal deoxyribonucleic acid and it uses the volcanic ash, bentonite, as a vehicle for precipitating the reactants. The results of this test are not identical to the conventional lupus cell preparation since they do not so far show significant cross-reaction with patients with rheumatoid arthritis. An extensive survey among non-lupus patients revealed almost no false positives. Thus, a positive DNA-bentonite test strongly indicates the diagnoses of systemic lupus erythematosus. This development will have wide application because of its high specificity and ease of performance. At a basic level, it appears to reveal a hitherto unidentified reaction of lupus serum. Many consider that this and the other reactions of lupus serum are indicative of a complex autosensitization to the hosts own nuclear components.

BIOLOGY OF VIRUSES

Significant scientific findings in the biology of viruses represent the beginning of systematic elucidation of individual facets of larger problems and the development of technical and procedural tools for future use. Under the former come the studies indicating two mechanisms of virus-cell association after adsorption, the mode of action of certain virus inhibitors, the intracellular carrier infection with typhoid organisms and their susceptibility to antibiotics, the localization of increased metabolic activity in tumor virus infected cells to perinuclear microsomes, and the resistance of ICM virus carried in tumor transplants to the immune mechanisms in vivo. Technical developments include development of methods for continuous observation of cells in tissue culture by phase contrast microscopy and the better preservation of metabolic activities of subcellular elements.

CELL BIOLOGY The work of the Laboratory of Cell Biology is developing along several lines. The seven "non-essential" amino acids, which the cell can synthesize in amounts sufficient for optimal growth, have been found to fall into three sharply defined groups. Aspartic acid, asparagine, glutamic acid and proline derive their carbon almost wholly from the glutamine of the medium. Of the remaining three non-essential amino acids, serine and glycine are metabolically quite distinct from alanine. When cells are grown in a glucose-free medium on ribose and pyruvate, the serine and glycine derive almost wholly from ribose, and the alanine almost wholly from pyruvate. The α-amino group of all the amino acids derives directly from that of glutamic acid. A large number of other transaminases known to be present in these cells are therefore physiologically inactive in these cell cultures.

A paradoxical finding of major interest is the fact that certain growth factors are rigorously essential for growth, despite the fact that they can be synthesized by the cell in amounts which should suffice for survival and sustained multiplication. One is inositol, which the cells can make from glucose; a second is cystine, which the cells make from methionine and serine; and certain mouse cell lines require pyruvate, despite the fact that the cells carry out active glycolysis, and are thus continuously making large amounts of pyruvate.

One cell line has been found which does not require added inositol, and which actually liberates small but significant amounts into the medium. This cell, growing on one side of a cellophane membrane, releases sufficient inositol into the medium to permit the growth of a second inositol-dependent strain, growing on the other side of the membrane. This example of parabiosis may serve as a useful model for the complex metabolic inter-relationships of different cells and different organs in vivo.

The puzzling nutritional requirement for serum protein by almost all cell lines, despite the fact that this protein is not used to a significant degree for the synthesis of cell protein, is now nearing solution. Human and animal cells are being grown in a protein-free medium separated by a cellophane membrane from the same medium supplemented with serum protein and proteolytic enzymes. It is obvious that essential growth factors are being provided which are either initially bound to the serum protein, or are formed from it on proteolysis. The identification of the specific enzymes concerned, of the types of protein which can release the active growth factor(s), and its chemical identification, are under continuing study.

BIOSYNTHESIS OF VIRUS

The second major line of investigation concerns the biosynthesis of the virus macromolecules, with particular reference to the precursors used by the cell, and the time course of their synthesis relative to the appearance of mature virus. These studies have been made possible by the use of highly radioactive precursors, and the development of techniques for the rapid purification of large amounts of poliovirus, grown in suspension cultures of HeLa cells. The results to date indicate that most of the viral protein and nucleic acid are synthesized at about the same time, simultaneous with the appearance of mature infectious virus. Both the viral protein and nucleic acid are synthesized from the small molecular weight components of the acid soluble pool of the cell, rather than by the breakdown of the preformed cellular protein and nucleic acid. In the case of vaccinia virus, however, most of the nucleic acid is synthesized within six hours after infection, at a time when only small amounts of mature virus have appeared, and approximately six hours before virus formation is completed. The significance of these findings is under continuing study.

SUMMARY OF RESEARCH PROGRAM
1959 ANNUAL REPORT
LABORATORY OF CLINICAL INVESTIGATION
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

GENERAL TRENDS

The laboratory and its associated clinical service have achieved considerable stability of function in the few years since they were established. Patient referrals are more regular and consistent, both locally and from distant areas, and valuable records of patients with illnesses of special research interest have been accumulated. For example, there are about 30 patients with cystic fibrosis of the pancreas, 10 with hypo- or agammaglobulinemia, 30 with disseminated lupus erythematosus, several salmonella carriers, and numerous cases of fungus infection available for recall when research interest requires it.

As with all evolving and developing programs, however, some old problems remain and new ones have arisen which require solution. The most important of these is undoubtedly the lack of intermediate and senior staff members. At present Dr. John Utz and Dr. Vernon Knight are the only staff members who have completed conventional training in internal medicine, while Dr. Henry Beye, with certification in preventive medicine, has been primarily trained in parasitology and tropical medicine, combining epidemiology with a clinical background. With a potential service of 52 beds and a daily census of 38 to 42 patients, this staff is insufficient. It would not be an exaggeration of needs to consider that not less than 8 full-time investigator-clinicians, including at least two pediatricians be assigned to the laboratory. These would be in addition to 12 clinical associates. As a beginning, an effort is being made to find a pediatrician to head this section of our research program and an internist trained in allergy and immunology to take over this area. On the basis of the brief effort we have already made, it is apparent that it is difficult to find well qualified men in these clinical specialities who are also good investigators. It appears that salaries for such men in medical schools are increasing, making recruitment difficult. Another important source of such personnel may be the clinical associates. These men are of high caliber and it is planned to invite some of the more promising of them to remain with the laboratory for an additional year or two and from them a permanent staff member may become available. This program is just beginning and it is likely that 2 or 3 years will be needed to judge its effectiveness. It is the clinical director's view that the acquisition of highly qualified personnel would do more to increase the productivity of the laboratory than any other measure.

I have indicated that patient material is more readily available now than earlier. This has primarily resulted because of the continued solicitation for referral of the selected entities mentioned earlier. This experience has demonstrated, I believe, that the period required to achieve a continuing supply of a specific kind of clinical material may require years instead of months. It seems to depend also on the personal influence and reputation of the senior investigator in charge of the program. For example, Dr. Utz is now nationally known for his interest in fungus disease and recently there has been an increased referral of patients with fungus disease to him. Thus referral often seems to be to persons rather than the institution. This then relates problems of patient referral to problems/senior personnel recruitment. There is little question in my mind that if we had a good pediatrician and a few more men with long-term interests in special diseases, we would soon have more and better patient material for study.

This year for the first time we have initiated a program of block time off for clinical associates so they may take up laboratory investigation. At present, they have one-third of their first year off and about one-half of their second year for this purpose. All of us realize that this is insufficient to establish competence in laboratory methodology but it should assist us to select the two or three men for reappointment in subsequent years. This plan makes it necessary for us to have some laboratory space for these men, especially since we increased the number to 12. It also requires increased funds for their operation. These items will be requested for future budgets.

It may be observed that we customarily have a large number of research projects listed, some of which are completely inactive, or nearly so. This is necessary so that we may have a regular flow of patient material. When the nursing service and others in charge of patient care are prepared for about 40 patients, it is inefficient and hard on the morale of the staff to have fewer patients than this. Thus, by having a number of different projects a continuous flow of patients is possible. When one project is to be emphasized admissions under other titles are reduced. The previously mentioned lack of senior personnel has also limited the number of long-term projects and the consequent continuity of referral of patients they could provide.

An important problem in connection with the laboratory's program is that of tropical disease. There is a deep interest in problems in parasitology and tropical disease in the laboratory and in the Institute of Allergy and Infectious disease. There are several good reasons why we should have within the National Institutes of Health a good program of clinical investigation in this area. For a year or so Dr. Baye has made a strenuous effort to obtain patients for such studies but with limited success. He has made arrangements to screen stools from returned State Department personnel, missionaries, and others for case finding purposes.

This has led to the discovery of a number of cases of tropical parasitic illnesses each year. They are not usually severely ill and have represented a sporadic and undependable source of material. The expense of maintaining a screening laboratory for this modest return of clinical material needs evaluation. Recently, our scientific counselors strongly recommended the establishment of a clinical tropical disease unit in an endemic area. This logically led to NARU as a possible site. It would have the advantage of being in an area under U.S. control but adjacent to large areas where serious tropical illnesses are frequent. A pre-clinical unit is already established and working there. Moreover, selected interesting cases could be sent to the Clinical Center from there when special studies were needed. The establishment of such a unit would be a large undertaking but on a preliminary basis, there is much to recommend it. It will be a hard thing to accomplish because of the difficulty of getting qualified investigators, and the Clinical Director is firmly against any reassignment of the intramural staff at this time.

Another area of interest as a source of patients is Alaska where the Public Health Service hospitals can provide patients with infections common to that region. Conversations with those in charge of that service indicate a cooperative attitude and we are beginning to make specific arrangements for admission of some of those patients. Cases of tapeworm, a highly lethal form of hydatid disease, and bacterial infections may be brought here.

The leprosy hospital of the Public Health Service in Carville is also interested in a cooperative research program. This will be more thoroughly studied before any action is taken.

PERSONNEL

The position of Clinical Director was assumed by Dr. Vernon Knight on July 1, 1959. Dr. John P. Utz had been acting director in addition to his other responsibilities as Chief of the Infectious Disease Service in LCI. He continues in this latter capacity with somewhat greater time now available to devote to research.

Dr. Donald Raynoe rejoined LCI on July 1, 1959, and has spent most of his time evaluating a test for systemic lupus erythematosus.

Dr. Henry Beye has been on duty with the laboratory throughout the year, except for a period of about 2 months this fall when he made a tour of British Guiana and Venezuela to make a survey of filariasis for the Pan-American Sanitary Bureau.

Dr. Harry Stalmwen, Dr. Eakin Huff and Miss Rose Lieberman have continued their research in the laboratory throughout the year.

MAJOR RESEARCH ACCOMPLISHMENTS

With the increasing interest in infections due to fungi, it is noteworthy that important advances in treatment are being made in this Laboratory. The number of patients (40) at the Clinical Center now treated with amphotericin permits the conclusions that this drug is the first effective treatment for some cases of cryptococcal meningitis and is a superior therapeutic agent in blastomycosis and histoplasmosis. A still newer antibiotic drug, known only by code number, under exclusive study at the NIH has been demonstrated in Dr. Chester Emmons' laboratory to be even more effective than amphotericin in experimental infections in mice due to Histoplasma and Blastomyces. The use of this drug in 12 patients with a number of infections suggests that it may be superior in human infections due to these same fungi.

In studies of viral diseases, our interests are more and more focused on possible involvement of the kidneys in infection. In addition to clinical and laboratory studies, appropriate to the diseases (respiratory, aseptic meningitis, etc.), special efforts have been devoted to search for virus in urine. In the past 2 years, 6 enteroviruses have been found in urine of patients and 3 of these urinary isolates have been studied in animals. Viral content of mouse kidney at certain times after inoculation is of sufficient titer ($10^{2.5}$ to $10^{4.0}$/mg.) to be poorly, if at all, explained by possible concurrent viremia. On the other hand, recent herpes and vaccinia isolates from man have not been found in rabbit kidney following intravenous inoculation. Virologic studies of urine are similarly being performed to evaluate the significance of urinary cells containing inclusion bodies. Studies of 57 hospitalized patients with ECHO type 9 seen during the summer of 1958 have correlated virologic and clinical aspects of this disease.

One aspect of the alarming staphylococcal problem is under unique study here. Investigation into the production and inhibition of penicillinase have revealed that a defined media containing relatively few amino acids promotes the production of this enzyme--encountered invariably in resistant staphylococci isolated from man. Production of the enzyme has been shown to be independent of bacterial multiplication and to be inhibited by the use of analogues of some of these essential amino acids. Studies of another important bacterial infection in man, Salmonella, have centered around the carrier state, which complication continues to be a problem despite otherwise effective antibiotic treatment. Stones recovered at cholecystectomy in 11 patients have been found infected in every instance, whereas gallbladder wall, liver, and bile were less frequently culturally positive. As a result of these studies, 5 stones from 3 carriers were implanted into the gallbladders of 5 rabbits. Salmonella was subsequently and repeatedly isolated from stools for periods now exceeding 7, 6, 6, 4, and 2 months.

Extension of the technique devised in this Laboratory for the production of potent antibodies in ascitic fluid of mice have shown differences in response of some strains of inbred mice. In addition, plasma cell tumors have been induced by this method in 3 BALB/CAnN mice. This method has proved suitable for the production of viral antibodies at a great saving in time and number of animals necessary. Continuing studies of patients with lupus erythematosus and agamma-globulinemia have revealed that with a much more sensitive technique of measurement patients with the latter disease do have detectable antibody to a number of enteroviruses. This provides a partial answer to the intriguing observation that patients with this disease seem to handle most viral illnesses well and without recurrence--in contrast to their experience with bacterial disease.

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Serial No. NIAID - 10 (c)
1. ICI
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Office of the Chief

Principal Investigator: Dr. John P. Utz (To July 1, 1959)
Dr. Vernon Knight (July 1 - Present)

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	5.8
Professional:	.5
Other:	5.3

Project Description:

Objectives:

To develop, direct and coordinate the program of the Laboratory, as defined in the individual research projects; where necessary, to re-direct individual projects to meet current needs and advances in the field; to align clinical projects with patient availability; to provide an unexcelled standard of patient care for patients utilized in research.

Methods Employed:

Organize available staff and recruit qualified personnel, both professional and sub-professional, to develop the program and carry out its aims; close liaison with area medical societies, institutions and individuals for referral of patients whose diagnoses fall within the active or proposed disease research categories; the highest standards of patient care are maintained by selection of qualified physicians; the development of necessary policies and continued close and direct supervision of patient care activities; professional consultant services to other institutes in the area of infectious diseases; continued guidance of research projects undertaken by younger investigators; maintenance of staff morale.

Part B included Yes ☐ No ☒

Serial No. NIAID - 12[illegible]
1. LCI
2. Infectious and Pediatric Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Respiratory Viral Diseases

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. George M. Owen
Dr. David Rifkind (from 7/1/59)
Julius A. Kasel
Clarence F. Szwed

Cooperating Units: Laboratory of Infectious Diseases, NIAID

Man Years (calendar year 1959):
Total: 3.85
Professional: 0.65
Other: 3.2

Project Description:

Objectives:

1. To define clinical entities in relation to newly isolated respiratory viruses.

2. Further to define diagnostic criteria, pathogenesis, immune response, persistence, sites and effects on tissues of virus in certain infections of respiratory passages and mouth.

3. To study host-parasite relationships in reference to susceptibility to chronic or recurrent respiratory diseases.

4. To improve clinical laboratory techniques in the laboratory confirmation of respiratory viral disease diagnosis.

Methods Employed:

Results of clinical observations and procedures are correlated with bacterial, mycologic and viral isolations employing both animals and tissue culture techniques.

Patient Material and Major Findings:

1. The Virology Unit of the Infectious Disease Service examined a total of 2064 specimens from patients under study. Virus was isolated in 117 of these specimens, achieving etiologic confirmation of the diagnosis in a total of 81 patients.

2. In a study of respiratory illness in children, simultaneous infection with hemadsorption virus type 1 and a HeLa enterovirus (Pett) was demonstrated. These findings further emphasized the difficulty in ascribing respiratory disease to a virus and in characterizing the clinical spectrum of illness produced in man by a single viral agent.

3. From an outbreak of a respiratory illness among children on a pediatric service, an adenovirus was isolated which was antigenically related to types 9 and 15. The clinical aspects of viral infection of these patients hospitalized for other clinical problems were described. In one of the patients during this study infection with this virus was associated temporarily with recurrence of active rheumatic fever.

4. Employing the technique first described from this Laboratory by Lieberman, et al., potent viral antibodies were produced in ascitic fluid of mice in substantive amounts. This technique results in substantial savings of time and expense, allowing for use of fewer animals, and additionally is uniquely suitable for the study of antibody response in the single animal.

5. Adenovirus type 2 has been isolated from a patient with a severe respiratory disease characterized by clinical and electrocardiographic evidence of myocarditis. Such severe manifestation of adenovirus infection has not been reported to our knowledge.

6. Despite the fact that virus etiology of rheumatoid arthritis has been occasionally averred, no studies employing modern virologic techniques have been done of synovial fluid. Such a study was undertaken and the results published. No viral agents have been uncovered thus far.

7. The knowledge concerning mouth lesions due to viral agents has been summarized in a symposium.

8. The presence of a factor in tissue culture preparations infected with certain adenoviruses has been demonstrated to be capable of modifying human group O erythrocytes. This erythrocyte receptor modifying (ERM) factor is separable from the infectious virus particle. Preliminary evidence indicates that the mechanism of action of the adenovirus factor is distinct from that of the receptor destroying enzyme (RDE) produced by Vibrio cholerae. The modification of erythrocyte receptor sites may possibly be due to enzymatic action.

Significance to Microbiological Research:

Illustrates progress in study of acute and chronic respiratory diseases, which are prime unsolved problems in the infectious diseases field. The major advances in this field are being made in settings such as this, where the efforts of research laboratories in bacteriology, mycology and virology can be correlated with clinical material under ideal study conditions.

A major effort is marked toward clinical studies in relation to newer viruses uncovered by tissue culture techniques.

Proposed Course of Project:

Studies will continue as outlined under "Objectives" in patients referred to the Clinical Center and in other hospital or community surveys when indicated. This project should be expanded because of the great importance of careful clinical studies on patients with respiratory illnesses in order to link these illnesses with viral agents that are now being isolated in a number of laboratories.

Part 2 included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Cramblett, H. G., Kasel, J. A., and Utz, J. P.: Respiratory illness in children simultaneously infected with hemadsorption virus type 1 and a newly recognized HeLa enterovirus (HE). Pediatrics 24(2): 234-240, 1959.

2. Cramblett, H. G., Kasel, J. A., Langmack, M., and Wilken, D.: Illnesses in children infected with an adenovirus antigenically related to types 9 and 15. J. Pediatrics. In press.

3. Kasel, J. A., Lieberman, R., and Smith, H.: Production of viral antibodies in ascitic fluid of mice. Virology. In press.

4. Utz, J. P., Phelps, E. T., and Smith, L. G.: Rheumatoid arthritis: Viral studies of adult human synovial fluid and tissue using tissue culture techniques. The Bulletin 12(5): 198-200, 1959, Georgetown University Medical School.

5. Driscoll, E. J., Ship, I. I., Baron, S., Stanley, H. R., Jr., and Utz, J. P.: Chronic aphthous stomatitis, herpes labialis and related conditions. Combined Clinical Staff Meeting of the National Institutes of Health. Ann. Int. M. 50(6): 1475-1496, 1959.

6. Kasel, J. A., Rowe, W. P., and Nemes, J. L.: Modification of erythrocyte receptors by a factor in adenovirus suspension. Submitted to NIAID Editorial Committee.

Serial No. NIAID - 14(c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Staphylococcal Diseases

Principal Investigator: Miss Rose Lieberman

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 0.9
Professional: 0.1
Other: 0.8

Project Description:

Objectives:

Basic studies of the pathogenesis and immunology of staphylococcal disease which include:

1. Investigation of the components and products of staphylococcus contributing to its pathogenicity.

2. Development of techniques to provide tools to study mechanisms involved in the establishment of staphylococcal infections.

Methods Employed:

The following studies are being conducted employing a specific phage type, penicillinase producing, antibiotic resistant strain of Staphylococcus aureus recently isolated from an abscess in a patient.

1. Serological classification according to the somatic antigenic formulae of Oeding.

2. Investigation of properties including coagulase, hemolysins (alpha, beta, etc.) leukocidins, mouse infectivity, etc.

3. Separate production of rabbit immune serum and of mouse "immune" ascitic fluid to produce large quantities of antibodies to be employed in antibody fractionation studies.

4. Effects of environmental conditions (incubation temperature, age of culture, etc.) on the formation of somatic antigenic components.

5. Absorption of immune sera and "immune" ascitic fluid to remove specific antibody fractions.

6. Employment of immunoelectrophoretic, gel diffusion, quantitative precipitin, immunofluorescent, and passive protection techniques to study the effects of the removal of specific antibody fractions.

7. Use of physical and chemical methods to fractionate the antigenic components of staphylococcus including ultrasonication, ultracentrifugation, cell disruption by the French press, ethanol extraction, ammonium sulfate precipitation, etc.

8. Investigation of reactions of specific antigenic fractions with both the native and fractionated staphylococcus antiserum.

Major Findings:

Employing immunoelectrophoretic techniques to study the interaction of immune rabbit serum or "immune" mouse ascitic fluid with staphylococcus antigenic material, definite lines of precipitation have been observed. By elution, dilution and absorption procedures these lines of precipitates are being identified and defined. Sonicated aliquots of staphylococcus antigenic materials produced different patterns of reaction with the native immune serum than did the intact organism. This difference appeared to be due to the presence of other antigenic components and not to a quantitative difference of the same antigenic factors.

Significance to Microbiological Research:

Increase of both primary and hospital infections with antibiotic resistant strains of staphylococcus has become a serious public health problem.

Research into the pathogenesis, immunology and etiology of staphylococcal diseases is especially important because of the lack of any effective antimicrobials.

Proposed Course of Project:

To continue as outlined under objectives.

Part B included Yes ☒ No ☐

FY59-RRR
Individual Project Report
Calendar Year 1959

III. F. Honors, Awards, and Publications

1. Publications other than abstracts from this project:

1. Lieberman, E., Douglas, J. O. A., and Mankel, H.: Production in mice of ascitic fluid containing antibodies induced by Staphylococcus or Salmonella-adjuvant mixtures. J. Immunol. (In press)

2. Kasel, J., Lieberman, E. and Smith, H. A.: Production of viral antibodies in ascitic fluid of mice virology. (In Press)

3. Lieberman, E., Douglas, J. O. A. and Humphrey, E., Jr.: Production in mice of ascitic fluid containing high antibody titers to staphylococcus or salmonella. Fed. Proc. 18: Part I, No. 364, Mar. 1959.

[illegible]
1. NIH
2. Infectious and Pediatric
Disease Service
PHS-NIH 3. Bethesda, Maryland
Individual Project Report
Calendar Year 1959

Part A-

Project Title: Hepatitis and Mononucleosis

This project has been temporarily inactivated due to lack of patient material. If patients become available, the project will be reactivated.

Serial No. NIAID - IDSC)
1. LCI
2. Infectious and Pediatric Disease Services
3. Bethesda, Maryland

FNS-WIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Aseptic Meningitis.

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. Reyard S. Tynes (to 7/1/59)
Dr. George M. Owen
Julius A. Kasel
Clarence P. Sawed

Cooperating Units: Children's Hospital, Washington, D. C.

Man Years (calendar year 1959):
Total: 2.95
Professional: 0.55
Other: 2.4

Project Description:

Objectives:

a) To develop methods and use newly available methods to diagnose the large group of non-bacterial meningo-encephalitides.

b) To correlate etiological findings with detailed historical physical and clinical laboratory observations.

c) To further define existing etiological entities and to discover new etiological entities in this group of diseases.

Methods Employed:

Hospitalization of patients for clinical observation and laboratory studies. Laboratory procedures include: a) tissue culture and suckling mouse inoculation of material for virus isolation; b) appropriate serological procedures with acute and convalescent serum for the known viral meningo-encephalitides, Leptospira, Toxoplasma and infectious mononucleosis. A number of patients selected from other hospitals or surveyed groups will be studied similarly.

Recent Material and Major Findings:

1. The clinical, virologic and epidemiologic aspects of an outbreak in Washington, D. C., in 1957 of aseptic meningitis due to type III poliovirus has been prepared and accepted for publication in the American Journal of Hygiene. The details of this study were mentioned in last year's Annual Report.

2. In July, August and September of 1958 the Washington, D. C. area experienced an epidemic of aseptic meningitis due predominantly to ECHO 9 virus. The clinical and virologic aspects of illness were studied in 57 hospitalized patients and are being prepared for publication. Of special interest were the occurrence of muscle spasm in 25% of cases and muscle weakness in 4 patients; the increased frequency of peripheral leukocytosis; the high percentage of viral isolations from cerebrospinal fluid (74% of those tested); and the presence of virus in urine of 2 patients.

3. The aseptic meningitis cases of 1959 are similarly being studied. No particular type of virus has seemed to predominate this year, and analysis of data is proceeding.

Significance to Microbiological Research:

Non-bacterial meningitis and meningo-encephalitis is a common and often serious disease of whose etiology and pathogenesis there is insufficient knowledge. It often results in brain damage and behavior disturbances. Increased knowledge may also clarify the pathogenesis of the encephalitic complications of the otherwise minor diseases of children.

Proposed Course of Project:

Continue above studies.

Part B included Yes [X] No [ ]

[illegible]
[illegible] Project Report
Calendar Year 1957

Part I. Honors, Awards, and Publications.

Publications (other than abstracts) from this project.

1. Sindler, A. D., Walrath, S. L., Thrupp, L. D., Ivie, J. C., Fenwicke, D. L., and Perrott, M. H. Gormandizer type (L.t parasitic poliomyelitis in Louisiana, U. S. in 1957. Am. J. Hyg. In press.

Serial No. NIAID - LCI (c)
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Allergy-Immunology

Principal Investigator: Dr. John P. Nasou
Dr. Donald E. Kayhoe (From July 1, 1959)

Other Investigators: Miss Rose Lieberman
Miss Margret Huber

Cooperating Units: Laboratory of Infectious Diseases, NIAID;
The Laboratory of Immunology, NIAID;
Division of Biological Standards;
Laboratory of Viral Products;
National Heart Institute, Laboratory of Cellular Physiology and Metabolism, NIH; and
Georgetown University Medical Center Clinical Laboratories.

Man Years (calendar year 1959):

Total:	4.8
Professional:	3.1
Other:	1.7

Sub-project I: Diseases of Immune Etiology

Project Description:

Objectives:

1. To evaluate the role of immune serum globulins in host response to infectious diseases.

2. To evaluate the role of bacterial, fungal, or viral allergies in recurrent or chronic respiratory disease.

3. To determine the rate of metabolism of individual antibodies compared to that of gamma globulin, and to

investigate the mechanism whereby the reticulo-endothelial system differentiates between otherwise chemically homogeneous specific antibodies.

4. To measure, quantitatively, the rate of synthesis of specific antibody in the human during the primary and secondary immune responses.

5. To measure quantitative differences in the specific antibody response of acute leukemics, normal human volunteers, hypo and hyper-gammaglobulinemias.

6. To determine whether, in the presence of a specific antigenic challenge, the specific antibodies are selectively removed from the total exogenous gamma globulin injected into agammaglobulinemia patients.

7. To determine the relationship of hormones to the agamma-globulinemic state.

8. To determine the pathogenic significance of the abnormal gamma globulin of patients with systemic lupus erythematosus.

9. To reproduce lupus lesions in experimental animals.

10. To study the antinuclear, anti DNA and antinuclear protein factors found in the serum of patients with systemic lupus erythematosus and to evaluate the relationship of the titer of the substances to the clinical status of the patient.

Methods Employed:

Clinical studies supplemented with:

1. Plasma protein analysis by (a) electrophoresis both by paper chromatography and moving boundary technique, (b) chemical determinations, and (c) immunologic assays.

2. Immunization and testing of responses by serologic techniques or by challenge.

3. Antibody and total gamma globulin alteration measurement in agammaglobulinemic patients following hormone administration.

4. Pathologic study of animals following injection into the circulating blood of suspensions of L. E. cells and L. E. bodies.

5. Phase contrast microscopic study of the L. E. cell phenomenon.

Patient Material and Major Findings:

1. In cooperation with Dr. Howard Goodman of the National Heart Institute and Dr. Richard Malmgren of the National Cancer Institute, studies of lupus factor as measured by the nuclear proteins sensitized and red blood cell technique and the fluorescent antibody technique, and its clinical correlation have been conducted. This data is currently being prepared for publication.

2. The clinical application of the procedure devised by Mr. John Bozicevich of the Laboratory of Immunology, NIAID, the DNA-bentonite flocculation test, has been conducted within the framework of this laboratory. This diagnostic method for systemic lupus erythematosus has a great specificity. This data is currently being prepared for publication.

3. Certain water precipitable proteins of the serum of patients with collagen vascular diseases are providing interesting immunologic phenomena. These materials are being studied further.

4. We are continuing clinical studies of the patients with lupus erythematosus and agammaglobulinemia.

5. In cooperation with Dr. Samuel Baron and Dr. Eugene Barnett of the Division of Biologic Standards, the study of agammaglobulinemia patients and the presence of antibodies in their serum to the enteric viruses has been conducted by use of very sensitive techniques to detect these antibodies. It has been found that all of these patients have significant titers. Further studies are being conducted in this vein in the attempt to elicit antibody formation by means of polio vaccine in those agammaglobulinemics not showing a significant titer.

Significance to Microbiological Research:

Unchanged

Proposed Course of Project:

Studies will be continued in a wide variety of clinical disorders as to the pathogen-host factors involved in infectious disease.

Study of the present methods of clinical management of agammaglobulinemia patients especially as to the required frequency and dosage of gamma globulin injection and their management with antibiotics will be continued.

The immune mechanism of agammaglobulinemic patients will be studied further, especially as to their response to vaccine and other antigenic stimulation. Non-antibody factors in resistance to disease will also be studied.

Of a special importance is the continuation of the long term followup of patients with systemic lupus erythematosus. This group of patients has been tediously followed in the clinic and with frequent inpatient admission and represents an outstanding group in regard to long term followup. During the course of the coming year it is anticipated that the relationship of the clinical course to the level of lupus factor as detected by the bentonite flocculation test will be a major endeavor.

The pathologic significance of the L. E. cell, especially when found in diseases other than lupus, will be studied.

Sub-project II: Ascites in Mice

Project Description:

Objectives:

To determine the role of genetic factors in the production of ascites in different strains of mice by staphylococcus-adjuvant mixtures.

Methods Employed:

Differences in the ability to induce ascites in inbred and non-inbred strains of mice are presently being investigated.

(a) CAF_1, A/LN, BALB/c AnN, $CDBA/ZF_1$, and DBA/2JN strains of mice were preimmunized with horse serum and then were injected with staphylococcus-adjuvant mixtures to induce ascites. Various immunization schedules were employed. Reactions of mice to inoculations with ascites inducing mixtures as hypersensitivity, Arthus reaction, etc., are being investigated. In addition, yield of ascitic fluid, persistence of production of ascitic fluid, and titer of antibodies in ascitic fluid are also under study.

(b) Quantitative measurements of precipitins produced in the ascitic fluid of these different strains of mice are being done.

(c) Examination of the gross and microscopic pathology in the different strains of ascitic mice kept for varying periods of time will be performed.

Major Findings:

There is a marked difference in the response of some of the inbred strains of mice to an ascites inducing staphylococcus-adjuvant mixture than that shown by the non-inbred strain. The reaction is more violent, and it is difficult to obtain appreciable yields of ascitic fluid. In three of the BALB/cAnN mice, plasma cell tumors have developed.

Significance to Microbiological Research:

Evidence to demonstrate that the ascites inducing property of antigen-adjuvant mixtures may be genetically controlled in a specific strain of mouse would be valuable to the geneticists and scientists engaged in cancer research. If this procedure can induce plasma cell tumor formation in a genetically defined ascitic mouse this information can be of inestimable value.

Proposed Course of Project:

To continue as outlined under objectives.

Sub-project III: Interaction of Antibodies

Project Description:

Objectives:

To study the effect of combinations of antibodies in conferring protection and reconciling the results obtained with various theories of antibody synthesis.

Methods Employed:

(a) Salmonella enteritidis and Salmonella typhimurium antisera were separately produced in rabbits and the fifty percent protective titer estimated in passive mouse protection tests.

(b) Combinations of these Salmonella antisera, absorbed and unabsorbed were studied in passive immunization tests and the protection afforded mice was compared with that obtained when a single Salmonella immune serum was employed.

Major Findings:

Wherever combinations of Salmonella antibodies were employed an interaction of the antibodies was noted so that enhancement, addition or antagonism occurred.

Significance to Microbiological Research:

Interaction of antibodies that may be beneficial or deleterious to individuals previously immunized with another agent should be considered in every program of vaccination. Employment of gamma globulin in passive immunization may have similar implications.

Proposed Course of Project:

To continue as outlined under objectives.

Part B Included Yes [X] No [ ]

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Antibodies to enteroviruses in hyper-gammaglobulinemic patients. Barnett, Eugene; Baron, Samuel; Nason, John; and Utz, John: To be published in New England Journal of Medicine.

Serial No. NIAID - 18
1. LCI
2. Bacteriology Section
3. Bethesda, Maryland

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Part A:

Project Title: Enteric Diseases

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. Bayard S. Tynes (to 7/1/59)
Dr. Howard Kravetz (from 7/1/59)

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2.65
Professional: 0.85
Other: 1.8

Project Description:

Objectives:

Basic studies of the etiology, pathogenesis, and immunology of enteric diseases which include:

1. The evaluation of antibiotic immunologic and surgical therapy on the "carrier state" of salmonellosis.

2. Studies of cellular and humoral mechanisms in active Salmonella infections as compared to those present in the "carrier state."

Clinical studies: To investigate the factors that produce persistence of bacteria in some patients to test new antibiotics in such infections.

Methods Employed:

1. Complete bacteriologic and immunologic survey of enteric disease study patients.

   a) *In vitro* determinations of synergistic, or additive effects of combinations of antibiotics on organisms isolated from "carriers."

b) Determination of gamma globulin, electrophoretic pattern, and agglutinating antibodies in acute and chronic enteric disease states.

c) Cholecystectomy or abscess irradication as indicated.

Patient Material and Major Findings:

1. Seventeen Salmonella carriers (15 S. typhi, 1 S. typhimurium, 1 S. schoenmülleri) have now been studied clinically and bacteriologically. After failure of antibiotic therapy maintained for at least 3 weeks, 11 patients had cholecystectomy and each was found to have gallstones. Fluorescein-tagged antibody studies of the gallbladder wall failed to show bacteria in 9 of 9 specimens. Cultural studies of operative specimens showed Salmonella in 1 of 5 liver biopsies, 6 of 11 bile specimens, and in wall of 5 of 11 gallbladders. Gallstones of all 11 patients showed Salmonella on culture.

2. When it began to appear that stones were consistently positive for Salmonella, 5 stones from 3 carriers were implanted into gallbladders of 5 rabbits. Salmonella were subsequently and repeatedly isolated from stools for periods now exceeding 7, 6, 6, 4, and 2 months. This material has been accepted for publication.

3. A rarely encountered infection with Fusobacterium polymorphum was investigated during the past year. Septicemia was established by 4 positive blood cultures and a positive bone marrow culture. Illness was characterized by high fever, shaking chills and drenching sweats, leukocytosis, anemia, elevated erythrocyte sedimentation rate and abnormal liver function tests were present. Liver biopsy showed focal necrosis and acute inflammation. Tetracycline therapy was associated with clinical recovery, negative blood cultures and return to normal of laboratory findings. Only 5 other cases culturally proved were found in the medical literature. Analysis of these and an additional 14 cases of septicemia not culturally proved showed striking similarities, chief among which are gastrointestinal or pulmonary site of origin, metastases to liver or brain, and poor prognosis without antibiotic therapy. This case and review of the literature has been prepared for publication.

Significance to Microbiological Research:

Outbreaks of salmonellosis represent a serious public health problem. The typhoid and other Salmonella "carrier states" are responsible for spread of the more virulent Salmonella to man, and pose a serious problem. There is no standardized therapy for the "carrier state."

Proposed Course of Project:

To continue as outlined under "Objectives."

Part B included Yes / / No /X/

Serial No. NIAID - A[illegible]
LCI
2. Parasitic Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Clinical Investigations on Helminthic Infestations

Principal Investigator: Dr. Henry K. Beye, Dr. Leon Jacobs, Dr. [illegible] Coatney

Other Investigators: Dr. James Kirby (to 7/59), Dr. Charles Brooks (to 4/59), Dr. Steven Schenker (since 7/59), and Elizabeth Guinn

Cooperating Units:
(1) Laboratory of Immunology, NIAID
(2) Parasitic Disease Service, LCI, NIAID
(3) Laboratory of Parasitic Diseases, NIAID
(4) Laboratory of Parasite Chemotherapy, NIAID
(5) Medical Division, Department of State, Washington, D. C.
(6) British West Indian Labour Organization

Man-Years (calendar year 1959)

Total:	2.65
Professional	1.20
Other	1.45

Project Description:

Objectives:

The objectives of this project are to conduct specific clinical investigations leading to improved diagnostic techniques, better understanding of host parasite relationships, more accurate descriptions of disease spectrums, and the elucidation of the pathogenesis of the disease processes, in helminthic infestations. These are integrated insofar as possible with basic laboratory interests and will be more specifically described as sub-projects related to I. Filariasis; II. Intestinal Nematode Infestations; III. Trematode Infestations and IV. Infections due to Cestodes.

Methods Employed:

Methods whereby clinical investigations in helminthic diseases can be effectively utilized in the attainment of these objectives are administrative and technical.

Administrative aspects are chiefly related to the two major problems of (1) helping promote an environment whereby basic laboratory competencies are sharply focused on human host-parasite relationships in infection and disease, and (2) securing suitable candidates for clinical investigation in these fields of interest.

During 1959, the Sections of Clinical Investigation have been created in the Laboratory of Parasite Chemotherapy, and also in the Laboratory of Parasitic Diseases. Administrative mechanisms were created for the coordination of these with the activities of the Parasitic Disease Service, Laboratory of Clinical Investigation. Although this arrangement is still in its developmental stages, there are indications of its effectiveness. Recently, great progress has been made toward securing suitable candidates for clinical investigation in tropical and parasitic diseases, and although a number of administrative problems remain unsolved, prospects are very hopeful for great improvement in our patient procurement problem. Institute funds are now available for the transportation of selected research candidates, while closer liaison has been established with foreign institutes interested in clinical investigation.

Existing methods for patient procurement utilized in 1959, consist of physician referrals, a cooperative arrangement with the Medical Division, Department of State, whereby returning State Department employees are screened for the presence of intestinal parasites, and an arrangement with the British West Indian Labour Organization whereby we can use British West Indian Agricultural Migratory Workers whenever indicated.

Educational activities indirectly related to patient procurement consisted in lectures and talks at George Washington University, Georgetown University, The Naval Medical Research Center and Walter Reed Army Medical Center. In addition, State Department physicians going to foreign areas spent from a few days to three weeks training in our Laboratory. A team sent to Cambodia under the sponsorship of WHO/FAO received their orientation in tropical diseases in our Laboratory. A scientific exhibit on intestinal parasitism among State Department employees was exhibited at the Scientific Assembly of the D. C. Medical Society held in Washington, D. C., November 1959. This was based on an analysis of 1,453 persons surveyed. During the past year, tabulations were almost completed on 5,000 additional epidemiologic questionnaires through the employment of students during the summer months. The Laboratory examined 5,900 stool specimens for screening or research purposes.

I. Filariasis

Objectives:

1. To clarify factors involved in host reaction and the existence of hypersensitivity in persons infected with *W. bancrofti*.

2. To elucidate the mechanisms of action of "Hetrazan" on microfilariae and possible effects on adult worms.

3. To determine precipitating factors in the production of attacks of filarial lymphangitis.

4. To explain the mechanism of periodicity of microfilariae.

5. To determine if transmission of the disease is occurring in the United States.

6. To develop procedures and methods whereby basic studies can be done more effectively.

7. To study pathogenesis and experimental therapeutics of elephantiasis.

8. To find more effective anti-filarial drugs.

Methods Employed:

During 1959 three of the four British West Indian Migratory Laborers found to be infected with Wuchereria bancrofti and/or Mansonella ozzardi were admitted for study. In addition, 2 young girls from French Equatorial Africa infected with Loa loa and Acanthocheilonema perstans were under observation. At the present time a patient with severe elephantiasis from Ceylon is under investigation. Each of these patients have complete basic work-up including day and night study for microfilaria, periodic X-rays, and numerous blood studies. Additional special studies are then carried out depending upon the results and where the candidate will best fit into a protocol of special studies devised to meet the objectives as listed above.

Patient Material and Major Findings:

Our series of filariasis patients at the Clinical Center now totals 14, 6 of which were studied during 1959. Interesting and significant findings are as follows: (1) Transmission can occur at low levels of microfilarial density, as for example, in the range of less than 1 microfilaria per 20 cubic mm peripheral blood, (2) Mansonella ozzardi does not respond to diethylcarbamazine therapy to the same degree as Wuchereria bancrofti, (3) tetracycline is concentrated in adult Loa loa worms as well as in Dirofilaria immitis and Ascaris lumbricoides. In patients infected with Loa loa the site of the adult worm can be seen when the patient is placed under ultraviolet light.

Significance to Bio-Medical Research:

The program is of considerable interest to the Institute for a number of reasons. The fact that carriers are in the United States as well as suitable mosquito vectors could conceivably help the infection become established within the United States. An estimated 309 million people in the world are infested with one or another of the species of human filariasis parasites and the United States is committed to the expenditure of other rather large sums of money in other areas of the world, in programs aimed toward the eventual control of filarial diseases. The specific results listed are of considerable significance. The demonstration of infectivity of human beings with low microfilarial density is of extreme interest in consideration of any control program, and studies are now in progress to investigate the effects of diethylcarbamazine administration in carriers of low microfilaria density. A demonstration of the phenomenon of tetracycline concentration in filarial worms has much potential interest and may be a clue to more effective treatment methods than the use of diethylcarbamazine.

Proposed Course of the Project:

In the past few months, arrangements have been instigated which will make possible the study of additional people with filariasis at the Clinical Center, and it is believed that the follow-up of tetracycline phenomenon in filariasis patients is a most promising lead. In line with past plans, it is hoped to apply immunological techniques to the study of various filarids within the next year. The progress to be made in this project depends upon the development of supporting technical staff, liaison and arrangements with foreign institutes and firm collaboration with the basic laboratory.

II. Intestinal Nematode Infestations

Objectives

1. To correlate clinical manifestations and pathology during the various phases of the life cycle of nematodes in the mammalian host, especially man.

2. To describe their role in the production of disease.

3. To evaluate the efficacy of established or new anthelminthics.

4. To investigate differences in species in terms of their clinical attributes.

5. To improve diagnostic techniques.

6. To elucidate the extent and severity of infestation in the United States.

Among intestinal nematode infestations, the program embraces those species whose life cycles include a period of tissue invasion and pulmonary migrations (Ascaris lumbricoides, Strongyloides stercoralis, and hookworm) as well as those species which do not exhibit a pulmonary cycle (Enterobius vermicularis and Trichuris trichiura). Reactions of the "tissue larva migrans" type are also of interest.

Methods Employed:

Parasitic screening of State Department employees returning to the United States and of patients referred by private physicians or governmental organizations; routine stool examinations on inpatients under study in LCE, laboratory diagnostic techniques, immunological studies, and therapeutics, especially with diathiazanine iodide and luethione.

The British West Indian Migratory workers provided an additional source of patient material.

Patient Material & Major Findings:

1. Trichostrongylus infected patients: Many of the State Department employees returning to the United States have been found passing eggs of this parasite in their stool. Clinical and laboratory data are lacking on this parasitic infection, especially in regards to the correlation between the number of eggs per gram of stool and the possible number of parasites harbored by the host and the pathogenesis they may be producing. Furthermore, appropriate therapeutic measures against this parasite are unknown. Finally the species of Trichostrongylus observed in these patients cannot be determined until the adult worms are recovered.

2. Parasites among the British West Indies Workers: In a study among the British West Indians workers blood was taken from 470 individuals and examined for malaria and filariasis. Stool specimens from 160 persons were examined for intestinal parasites.

The range in age of these workers is from 21 to 48 years. Eighty-four are from Jamaica, and the remaining 56 from 8 other islands in the British West Indies. It is of interest to note that hookworm was the most prevalent parasite, being found in 54.3% of the men. The percentages for other intestinal parasites in terms of frequency were: Endolimax nana 42.9%, Trichuris trichiura 33.8%, Entamoeba coli 25.8% Giardia lamblia 13.8%, Entamoeba histolytica 12.0%, Strongyloides 10 5%. Iodamoeba butschlii 6 4% and Ascaris lumbricoides 4.9%.

Significance to Bio-Medical Research:

Many aspects of intestinal nematode infestations can contribute to a better understanding of host-parasite relations and lead to alleviation of disease in the United States and abroad. The finding of a relatively high prevalence of Trichostrongylus infestation among State Department employees returning from the Middle East is of significance because this manifestation is rare among Americans as of present date. The parasitologic, clinical and epidemiologic characteristics of this infestation needs further clarification.

Within the next five years, world-wide programs against intestinal nematodes will probably be undertaken. Knowledge gained through the present programs can well be influential in planning these programs.

Proposed Course of Project:

Present plans call for the completion of the surveys involving sewage sludge this coming year. This interesting work was reported in 1957, but we were unable to carry out further studies in 1958. Work will be intensified on investigations with trichostrongylus, including trials of a new drug which seems effectual in the laboratory. If staff and facilities permit, fundamental studies on the mechanism of eosinophilia will be started. Patients suffering from Strongyloides and visceral larva migrans infestations will provide one source of patient material for these studies.

The British West Indian workers will provide patient material for further development of this project, and closer collaboration with the Laboratories of Parasite Chemotherapy and Parasitic Diseases will enable a more intensive study of intestinal nematode infestations and the human host.

III. Trematode Infestations

1. Schistosomiasis
2. Other diseases due to flukes

Objectives:

Besides schistosomiasis, this project is concerned with paragonimiasis and diseases due to various liver flukes. The aim is to study (1) systematic reactions to the infestations, (2) immunologic responses, (3) pathologic processes resulting from infestations, (4) evaluation of new or experimental prophylactic or therapeutic compounds, and (5) improvement of diagnostic techniques.

Methods Employed:

Field surveys of selected groups of people for trematode infestations, parasitic screening and work-up of patients referred by private physicians or governmental organizations for suspected trematode infestation, parasitological methods of diagnosis applied to feces, urine, bile, serologic studies, including appropriate skin tests, and reactions between ova and patient serum; clinical studies on proved cases; experimental therapy.

Patient Material & Major Findings:

Continued studies of the efficacy of Miracil D (Miloiddia), and its pharmacology indicate that it may be effective in the treatment of infestation with Schistosoma mansoni.

Significance to Bio-Medical Research:

With the great migration of persons into the United States from areas of schistosomiasis endemicity, and the increasing number of Americans going into infested areas, plus the demonstrated increasingly larger areas of the world in which infested snails are found makes schistosomiasis a disease of increasing importance to this country as well as to the world at large.

Treatment at the present time, largely by intramuscular and intravenous routes, is costly and dangerous. In areas where the danger of re-infestation is unlikely, such as the United States, it would be advantageous to have a potent orally effective agent for the treatment of early cases of the disease to prevent the later sequelae which may be fatal.

It is of interest that Miracil D has anti-leukemic properties and is being studied from this point of view. Data obtained in urinary excretion and toxicity contributes toward this new application of the drug.

Proposed Course of Project:

Continued efforts to evaluate new drugs with an emphasis on the pharmacological approach and thorough studies on the efficacy and toxicity of these drugs by long-term studies on persons not in endemic areas of the disease.

In association with the Laboratory of Parasitic Diseases, clinical studies will be undertaken to provide a better understanding of the pathogenesis of many of the manifestations of schistosomiasis.

IV. Infestations due to Cestodes

Objectives:

1. To determine the extent of cestode infestations in Americans and persons returning to the United States from other areas.

2. To study the signs and symptoms of the disease in otherwise healthy individuals.

3. To evaluate laboratory diagnostic procedures.

4. To evaluate various methods of therapy.

Taenia saginata (beef tapeworm and Taenia solium (pork tapeworm) are of principal interest. In infestations due to these worms, studies are centered chiefly on the effects that these parasites have on the physiology of the bowel and on the effect of therapy in the eradication of the parasites.

In the diseases in which the symptoms are due to tissue invasion by the parasite, investigation is directed toward determining the effect of the parasites on various organs involved.

The dwarf tapeworm, Hymenolepis nana and H. diminuta are of interest because they are especially refractory to therapy.

Methods Employed:

Patients are secured for study through the State Department referral method and the physician patient referral system as previously described. During the past year studies on the efficacy of intraduodenal quinacrine have been under way.

Patient Material & Major Findings:

To present date, a series of 20 patients have been treated with atabrine administered through a duodenal tube. This series is not large enough to serve as base data, for subsequent comparative studies using drugs which can be administered orally. To date, dithiasone iodide has been tried with inconclusive results.

Significance to Bio-Medical Research:

Infestations with these parasites, although rare in the United States, are difficult to treat. The diseases are quite common in other parts of the world, and could potentially become a problem in this country. Cases of Echinococcus granulosa, especially among the

Eskimos and Indians present diagnostic and therapeutic problems which, because of increasing work in the study of diseases among these people is becoming more and more appreciated. Large areas of South America are heavily infested with these parasites and in these areas they are of major importance, not only as a disease among the sheep in the area but among the people living there. The treatment at the present time is unsatisfactory. These diseases could become established in the sheep raising areas of the United States.

Proposed Course of Project:

If time and staffing permit, surveys among sheep raising areas of this country may be of great value in determining the importance of the disease in this country at the present time.

Part B included: Yes [x] No [ ]

(Attachment I)

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Baye, Henry K., Brooks, C., and Guinn, E.: Intestinal and blood parasitism among British West Indian Agricultural Laborers. Review of protozoan and helminthic infestations among migratory agricultural workers. (Presented at Amer. Soc. of Trop. Med. & Hygiene Meeting, October 28, 1959.) (In press).

2. Baye, Henry K.: Review of the problems of filariasis and the filariasis program in British Guiana. (Submitted to WHO for publication).

3. Baye, Henry K.: Filariasis and the filariasis program in Surinam. (Submitted to WHO for publication).

4. Baye, Henry K. and Wright, W. H.: The National Filarial Control Program (NFCP) of India: Investigative Challenges. Bulletin National Society of India for Malaria & Other Mosquito-Borne Diseases. (In press).

Serial No. NIAID - 20 (c)
1. LCI, LTC, LPD
2. Parasitic Disease Service
Laboratory of Parasite Chemotherapy
Laboratory of Parasitic Diseases
3. Bethesda, Maryland

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Calendar Year 1959

Part A.

Project Title: Clinical Investigations on Protozoan Infections

Principal Investigator: Dr. Manny R. Baye Dr. Leon Jacobs and Dr. Robert Costney

Other Investigators: Dr. Donald Kayhoe Dr. James Kirby (to 7/59), Dr. Charles Brooks (to 4/59), Dr. Steven Schenker (since 7/59), Elizabeth Guima

Cooperating Units:
(1) Laboratory of Immunology, NIAID
(2) Parasitic Disease Service, Laboratory of Clinical Investigation, NIAID
(3) Laboratory of Parasitic Disease, NIAID
(4) Laboratory of Parasite Chemotherapy, NIAID
(5) Medical Division, Department of State, Washington, D. C.
(6) British West Indian Labour Organization

Man Years (calendar year 1959)
Total: 3.40
Professional: 2.35
Other: 1.05

Project Description:

Objectives:

The objectives of this project are to conduct specific clinical investigations leading to (1) improved diagnostic techniques (2) better understanding of host parasite relationships (3) more accurate descriptions of disease spectrums, and (4) the elucidation of the pathogenesis of the disease processes, in protozoan infections. These investigations are integrated insofar as possible with basic laboratory interests and will be more specifically described as sub-projects related to: I. Amebiasis; II. Blood and Tissue Protozoan Diseases; III. Trichomoniasis and IV. Toxoplasmosis.

Methods Employed:

Methods whereby clinical investigations in protozoan diseases can be effectively utilized in the attainment of these objectives are administrative and technical.

Administrative aspects are chiefly related to two major activities: (1) helping promote an environment whereby basic laboratory competencies are sharply focused on human host-parasite relationships in infection and disease, and (2) securing suitable candidates for clinical investigation in these fields of interest.

During 1959, the Sections of Clinical Investigation were created in the Laboratory of Parasite Chemotherapy, and also in the Laboratory of Parasitic Diseases. Administrative mechanisms were established for the coordination of these with the activities of the Parasitic Diseases Service, Laboratory of Clinical Investigation. Although this arrangement is still in its developmental stages there are indications of its effectiveness. Recently, progress has been made toward securing suitable candidates for clinical investigation in tropical and parasitic diseases, and although a number of administrative problems remain unsolved, prospects are very hopeful for great improvement in patient procurement. Institute funds are now available for the transportation of selected research candidates, while closer liaison has been established with foreign institutes interested in clinical investigation.

Existing methods for patient procurement utilized in 1959, consisted of physician referrals, a cooperative arrangement with the Medical Division, Department of State, whereby returning State Department employees were screened for the presence of intestinal parasites, and an arrangement with the British West Indian Labor Organization to use British West Indian Agricultural Migratory Workers whenever we so desire.

Educational activities indirectly related to patient procurement consisted in lectures and talks at George Washington University, Georgetown University, the Naval Medical Research Center and Walter Reed Army Hospital. In addition, State Department physicians going to foreign areas spent from a few days to three weeks training in our Laboratory. A team sent to Cambodia under the sponsorship of NEDICO received their orientation in tropical diseases in our laboratory. A scientific exhibit on intestinal parasitism among State Department employees was exhibited at the Scientific Assembly of the D. C. Medical Society held in Washington, D. C., November 1959. This was based on an analysis of 1,453 returning State Department personnel. During the past year, tabulations were almost completed on 5,000 additional individuals through the employment of students during the summer months. The Laboratory examined 5,694 stool specimens for screening or research purposes.

I. Amebiasis

Objectives:

(1) To improve and standardize diagnostic techniques for Amebiasis, (2) to determine the prevalence of the parasite in persons residing in and returning to the United States, (3) to study the possible role of strain differences, altered host resistance, associated bacterial flora and the effect of nutrition on the pathogenicity of the parasite, and (4) to evaluate under adequate controls, the efficacy of certain antiamoebic drugs.

Methods Employed:

Patients who have been shown to have amebiasis are accepted for study. A history is obtained with emphasis on exposure or symptomatology that may have been a result of parasitism. A complete physical examination is done, including proctosigmoidoscopy in selected patients. In addition to the routine laboratory studies, special studies are done of renal and hepatic function as well as serological tests. Upon completion of the basic studies, the patients are placed on chemotherapy post-treatment studies are made to determine any toxicity of the drugs and their efficacy. Patients are followed for one year whenever possible.

Patient Material and Major Findings:

Epidemiologic studies associated with screening of returning State Department personnel continued during this year, and as indicated this material covering 5,000 individuals is now being analyzed.

Research candidates with Entamoeba histolytica were handled mainly through the Admissions and Follow-up Department. Generally they expressed few major complaints, and demonstrated no abnormalities on physical examination. Laboratory studies were usually within normal limits.

During the first part of 1959, patients were treated with tetracycline and chloroquine, while during the latter part, they were treated with humamycin.

An analysis of the effectiveness of these regimes, excluding humamycin is in process. Generally speaking, tetracycline seems about 70% effective in producing a parasitologic cure.

Significance to Bio-Medical Research:

A clinical understanding of amebiasis including the clinical spectrum of disease and the pathogenesis of infection remain little understood. Well documented observations of infected individuals add considerably to our knowledge. The use of returning State Department employees has certain advantages, although these advantages have not been completely explored. One of these is the opportunity of being able to study E. histolytica strains from various parts of the world. Another is the opportunity to study the infection in individuals which are otherwise generally in good health - a condition which does not always exist in other parts of the world or with other population groups and where other infections, or dietary factors may complicate the observations. On the basis of these studies, patients seen at the Clinical Center do not have the serious manifestations often reported from other parts of the world, although the infection rate is approximately 5%. The pathogenesis of clinical manifestations or the relation between infection and disease remain to be elucidated.

Proposed Course of the Project:

During the next year, it is hoped to emphasize detailed studies of the individual patient, and if possible, to more accurately define some of the human factors in pathogenesis. If suitable candidates and staff are available such investigations would include the study of infected individuals under different dietary regimes observing changes in E. histolytica, intestinal flora, symptoms, and responses to treatment. The use of the intestinal biopsy may be incorporated into these studies. Epidemiologic information will probably be de-emphasized if after tabulations and analysis of present data, this seems to be indicated.

Efforts will continue toward improvements in diagnostic techniques. Much of the clinical investigation aspects of amebiasis will depend upon developments, leads, and findings coming from the basic laboratories.

II. Blood and Tissue Protozoan Diseases

Objectives:

(1) To improve diagnostic techniques, (2) to study the clinical and pathological manifestations of these diseases in man and their pathogenesis, (3) to evaluate the experimental therapeutic and prophylactic results and (4) to aid in the development of chemotherapeutic agents suitable for mass therapy. During 1959, activities toward these objectives have been almost entirely with malarial parasites and almost entirely performed upon volunteers at the Federal Penitentiary in Atlanta, Georgia.

Methods Employed:

Human volunteers at the Federal Penitentiary in Atlanta were preliminary screened by history, physical examination, chest X-ray and routine laboratory tests. Those giving evidence of being suitable candidates for investigations on malaria were included in the roster. Dr. Morton Gots of the Laboratory of Parasite Chemotherapy has maintained residence in Atlanta and has been responsible for the day to day observations on these groups of volunteers. Hospital and laboratory facilities at the Penitentiary as well as additional laboratory facilities in Atlanta, such as at Emory University, and the facilities at NIH have been available for both day to day patient care and research observations. The volunteers were separated into control and various groups depending upon treatment regimes under investigation. During the course of the individual experiments, the volunteers were usually bitten by Anopheles quadrimaculatus infected with the Chesson strain of Plasmodium vivax. Recently a Venezuelan strain of Plasmodium vivax has been utilized in these investigations.

Patient Material and Results:

In a study to determine the prophylactic and sporontocidal properties of primaquine, 15 white males were each infected with Chesson strain Plasmodium vivax by the bites of 10 heavily infected Anopheles quadrimaculatus mosquitoes on April 17, 1959.

These 15 volunteers were separated into groups of 5 men each and given drug as follows:

Group A. Primaquine, 30 mg. (base) weekly, beginning on day 0-1 and continuing on the same day once each week.

Group B. Primaquine, 3 mg. (base) daily, beginning day 0.

Group C. No drug.

All the volunteers in Group A showed parasites between day 9 and 13 after infection. All in Group B became positive day 11 to 12, and all in Group C exhibited parasites between day 8 and 13. All of the subjects in Groups A and B, except one, were treated with 600 mg. (base) chloroquine, single dose; parasitemia cleared promptly. Early treatment of volunteers in Group C was withheld in order to allow gametocytes to build up in the blood. On 11 May, mature gametocytes were plentiful in the circulating blood of four of these subjects. Clean mosquitoes were applied to each volunteer on that day and each subsequent day for a total of 12 days. Shortly after the second feeding, each subject received 3 mg. (base) primaquine and the same dose was repeated daily, after that day's feeding episode, for a total of 10 doses.

Mosquitoes allowed to take blood prior to primaquine therapy became infected. Mosquitoes fed on day 1 through 6 after primaquine therapy showed cysts on their guts. Salivary gland infections were demonstrated in mosquitoes fed prior to primaquine therapy. Due to mechanical and other difficulties in the insectary, mosquitoes fed after primaquine was begun did not live long enough to develop gland infections.

All 15 men in the first test, begun 17 April 1959, were treated with primaquine, 15 mg. (base) daily for 14 days. Four volunteers have relapsed, 18, 39, 74 and 114 days after the last dose of the drug. This further proof, if such were needed, that the recognized regime of primaquine treatment will not produce cure of all Chesson vivax infections.

Due to the 'feeling' in some quarters that 300 mg. (base) of chloroquine, single dose, weekly, is in excess of what is needed to give complete suppression, a study was set up to settle this point. Fifteen white males were selected; infection was by the bites of A. quadrimaculatus mosquitoes infected with the Chesson strain of P. vivax. After the initial infection there were to be subsequent biting episodes during the life of the test.

The volunteers were divided into three groups of 5 men each with extra controls added at each biting episode. Each group was handled initially as follows:

Group A. 150 mg. (base) single dose, weekly, beginning on day 0-1 and continuing on the same dose each week.

Group B. 75 mg. (base) as A, above.

Group C. No drug.

A second 'infection' was carried out (Groups A and B with the addition of two controls) on 0-3.

All the volunteers in Group A were protected 2, and possibly 3 in Group B have shown parasites. Of the 5 initial controls (Group C) only two had exhibited parasitemia, day 14 and 15, respectively, after the initial bite.

Significance to Bio-Medical Research:

Besides the continuing world-wide prevalence of malaria throughout the world, two recent developments have placed an urgency on new knowledge on the chemotherapy of these protozoan diseases. These developments have been first, the demonstration of Anopheles resistance to DDT, BHC, and other insecticides, throughout many parts of the world and second, the great emphasis now being placed on eradication of malaria

Many people feel that if this objective is to be accomplished, recourse to mass chemotherapeutic measures will be necessary under conditions existing in many parts of the world. Contributions which can be made in this area will have great significance. Study of other protozoan diseases, particularly Chagas', is of considerable pertinence although this disease has not yet been incorporated into our study program.

Proposed Course of the Project:

The project will undoubtedly be emphasized in 1960 primarily through greater joint participation with the Laboratory of Parasite Chemotherapy.

III. Trichomoniasis

Objectives:

The objectives in the past have been to determine the extent of trichomoniasis and Candida among the population of females working at the National Institutes of Health, to study effects on vaginal cytology which may occur as a result of the parasite; to gain epidemiologic data on the disease and to determine the role of the parasite in the pathogenesis of certain vaginal complaints. The efficacy of various drugs was also under study.

Patient Material, Results and Proposed Course of Project:

In 1959 clinical investigations in this area have been curtailed pending further basic laboratory knowledge. The present clinical investigation participates only by collecting specimens and relating these specimens to clinical manifestations for studies on pathogenicity on various human strains in mice.

The course of this project will be dependent upon laboratory interests and leads.

IV. Toxoplasmosis

Objectives:

(1) To study clinical and laboratory manifestations of the disease, (2) to evaluate diagnostic methods, (3) to study the immunologic response of the host to the parasite, and (4) to determine effective methods of therapy in patients with proven toxoplasmosis.

Methods employed:

Serological surveys and clinical studies on patients with symptoms suggestive of toxoplasmosis, or on patients with serological

evidence of the disease. In an attempt to more clearly delineate the manifestations of the disease in its multiple forms. Recently much effort has been expended in making physicians in the area aware of the symptomatology of the disease with the expectation of this resulting in additional patient referrals.

Patient Material and Major Findings:

Continuing studies on the two proved acquired cases of adult toxoplasmosis.

Significance to Bio-Medical Research:

Serologic and skin test surveys indicate that there is a high incidence of toxoplasmosis in man, yet clinical reports of the disease are relatively rare. There is much to be learned about the clinical manifestations of the disease and methods which may be employed in its diagnosis. Experience has shown that pathologic diagnosis is difficult even in patients in whom the diagnosis has been established by the recovery of the parasites by inoculation of tissues into laboratory animals. With the exception of chronic ocular toxoplasmosis, little is known of the chronic form of the disease in humans although the parasite seems to survive for long periods in laboratory animals. Studies by other investigators have suggested that the parasite may play a role in the production of myocarditis and chronic encephalitis.

Proposed Course of the Project:

This will be dependent upon suitable patient material and the development of epidemiologic mechanisms to bring early and chronic cases to our attention

Part 2 included: Yes [X] [ ]

(Attachment I)

Serial No. NIAID - 20(c)

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Guinn, E.: The use of the zinc sulfate centrifugation flotation technique as a routine diagnostic procedure. American Journal of Clinical Pathology, 1:87-88. January 1959.

2. Beye, H. K., Brooks, C., and Guinn, E.: Intestinal and blood parasitism among British West Indian Agricultural Laborers. Review of protozoan and helminthic infestations among migratory agricultural workers. (Presented at Amer. Soc. of Trop. Med. & Hygiene Meeting, October 28, 1959. (In press).

PHS-NIH
Individual Project Report
Calendar Year 1959

Project Title: Cystic Fibrosis of the Pancreas

Principal Investigator: Dr. Lowell K. Good

Other Investigators: Dr. George Bryan
Dr. George Owen

Cooperating Units: Children's Hospital of Philadelphia and University of Pennsylvania Medical School

Man Years (calendar year 1959):
Professional: 1.9

Project Description:

Objectives and Methods Employed:

I. Clinical Investigation

A. Work is in progress to study the effect of duodenal fluid replacement from a normal subject to a child with moderate clinical manifestations of fibrocystic disease of the pancreas. The duodenal contents are obtained through a nasal duodenal tube from a normal subject at specific intervals throughout the day and transferred immediately to a child with fibrocystic disease of the pancreas by way of a nasal duodenal tube. Certain end points used to detect activity of this replacement therapy are:

1. The change in sodium chloride concentration of sweat.

2. A qualitative change in the presence of the water insoluble fraction in stool or duodenal fluid.

3. The change in the clinical picture of course of the disease.

This type of replacement therapy may be the only means of demonstrating biological activity of a substance which may be present in normal duodenal fluid and not in the duodenal contents of children with fibrocystic disease of the pancreas. This work has not been done before. The closest work of a similar nature was associated with an attempted replacement using whole pancreas or pancreatic extracts.

B. An evaluation of intermittent antibiotic therapy in children with fibrocystic disease of the pancreas by a double-blind technique.

C. Evaluation of the roll of viruses during the acute exacerbations of fibrocystic disease of the pancreas. The acute exacerbations refer mainly to changes of the pneumonitis.

D. Determination of antibiotic levels in pulmonary secretions collected at time of autopsy and bronchoscopy.

E. The study of antibiotic absorption of the GI tract in fibrocystic disease of the pancreas.

F. The study of certain body fluids for the presence of the water insoluble fraction as described by Dische. These body fluids are stools, amniotic fluid, cerebrospinal fluid, serum, urine and saliva.

G. Determination of the salt concentrations in amniotic fluid from a mother whose child is shown to have fibrocystic disease of the pancreas.

II. Animal Experimentation:

The objective of this work is to determine changes in the functional activity of the sweat glands in the hairy skin of the dog, after alterations in pancreatic function prompted by pancreatic duct ligation or total pancreatectomy. The pre- and postoperative concentrations of sodium chloride in the sweat may reflect some relation between the pancreas and the sweat glands. This perhaps could in turn be related to dysfunction of the pancreas and sweat glands in fibrocystic disease of the pancreas.

III. Immunological and Electrophoretic Studies:

A. Immunological characterization of the water insoluble fraction described first by Dische.

The following substances were used in this characterization:

1. Duodenal fluid

2. Saliva

3. Water soluble fraction

4. Water insoluble fraction

5. A benzene alcohol extract of duodenal fluid.

The following studies were done using the above substances:

1. Hemaglutination inhibition with influenza PR8 and adenovirus
2. Agar gel diffusion
3. Precipitin reaction
4. Agglutination reaction
5. Complement fixation tests
6. Skin tests

B. Paper electrophoresis of serum and duodenal fluid from patients with fibrocystic disease of the pancreas.

The mucoprotein described by Dische appears to be present in the serum and mucoprotein in general migrate with the alpha 2 globulin fractions. An attempt is being made to determine if alpha 2-globulin in serum is greater in children with fibrocystic disease of the pancreas in contrast to normal or noncystic children. No conclusive data is available at this time.

Patient Material and Major Findings:

A group of approximately 35 children with fibrocystic disease of the pancreas is being followed. Patients on the wards are used to obtain clinical non-cystic specimens.

Significant results of the above work indicate that:

(1) Antibiotic absorption of the GI tract in children with fibrocystic disease of the pancreas does not differ greatly from absorption in normal subjects.

(2) The water insoluble fraction has been demonstrated in amniotic fluid, saliva, stool and urine in children with fibrocystic disease of the pancreas as well as of the duodenal fluid.

(3) The water insoluble fraction is also demonstrated in the stool and duodenal fluid of several normal subjects or non-cystic subjects.

(4) The salt concentration of the amniotic fluid from a mother whose child was later shown to have cystic fibrosis was equivalent to the normal values established by Canterrow.

(5) The minimum amount of sweat required for a significant determination of the sweat and sodium chloride using iontophoresis and gauze collection is 100 mg.

(6) It has also been shown that the collection of sweat by iontophoratic stimulation with subsequent collection per 2 hour period is not significantly different from sweat collection in the same fashion but without iontophoretic stimulation and with collection for a much longer period of time, namely 24 hours.

(7) Although the water insoluble fraction is described as a mucoprotein it has no effect upon hemaglutination using influenza PR8 and adenovirus 9.

(8) No activity has been demonstrated using hemagglutination reactions, flocculation techniques and agar gel diffusion methods.

(9) A precipitin reaction demonstrates a specific and moderately sensitive test which may have definite merit in the diagnosis and prognosis of fibrocystic disease of the pancreas.

(10) A skin test using the water insoluble fraction seems to in part reflect the quantity of serum gamma globulin.

Proposed Course of Project:

On the basis of work done in this Institute on fibrocystic disease during the past year it seems reasonable to suggest that a future course of research in this Institute should be directed toward serological studies and normal duodenal fluid replacement. Recent results indicate that further studies in this area may reveal basic etiological information as well as practical diagnostic and therapeutic data.

Part B included Yes [ ] No [x]

Serial No. NIAID - 22(c)
1. LCI
2. Biochemistry and Immunochemistry Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemical Studies

Principal Investigator: Dr. Harry G. Steinman

Other Investigators: Dr. Eakin Huff

Cooperating Units: None

Man Years (calendar year 1959):

Total:	4.0
Professional:	2.0
Other:	2.0

Project Description:

Objectives:

To study the biochemistry of pathogenic micro-organisms with particular emphasis on their carbohydrate metabolism and on the nature of antibiotic resistance.

Methods Employed:

1) Carbohydrate Metabolism
The biosynthesis of polysaccharides peculiar to bacterial cell wall material is being investigated with special attention to the role of certain three-carbon precursors.

2) Nature of antibiotic resistance
An intensive study is being made of the factors controlling the formation of increased levels of penicillinase when pathogenic Staphylococcus aureus is exposed to penicillin.

Major Findings:

1) Carbohydrate Metabolism
Previous investigations on the metabolism of 1,2-propanediol phosphate and on the separation of mixtures of acetol, lactaldehyde, pyruvaldehyde, and pyruvic acid on bisulfite ion exchange columns were published in 1959.

By means of the ion exchange method it was possible to identify the enzymatic oxidation product of 1,2-propanediol as lactaldehyde. This result and the results of other studies on the enzyme preparation from rabbit liver which oxidizes 1,2-propanediol were presented at the Federation Proceedings, April, 1959. Publication of these studies is now pending the completion of the present related studies on the metabolism of lactaldehyde. The pure D and L isomers of 1,2-propanediol and lactaldehyde have been prepared. Kinetic studies of the oxidation of the two isomers of 1,2-propanediol by rabbit liver extracts has suggested that a different enzyme is involved in the oxidation of L-1,2-propanediol than involved in the oxidation of D-1,2-propanediol. Attempts at separation of these two enzymes are in progress. Crystalline horse liver alcohol dehydrogenase is more active on the L-isomers of 1,2-propanediol and lactaldehyde than the D-isomers. This is the first time that an alcohol dehydrogenase has shown a steric preference for substrates that differed in configuration on the carbon adjacent to the one undergoing oxidation. These specificity studies will be extended to the alcohol dehydrogenases of Pseudomonas aeruginosa, S. aureus, and other organisms.

As was mentioned in the last report (see Project Report NIAID-22 (c), 1958) it was proposed that an attempt be made to determine if lactaldehyde is an intermediate in the biosynthesis of rhamnose. Such an attempt has been made. Several strains of P. aeruginosa were obtained from Dr. Verder and tested for their ability to synthesize rhamnose. Extracts of two of the strains which produced large amounts of rhamnose were unable to synthesize rhamnose in the presence of lactaldehyde and dihydroxyacetone phosphate - the proposed precursors of rhamnose. Further studies on the biosynthesis of rhamnose by these organisms are planned.

2) Nature of antibiotic resistance

The previous findings described in Project Report NIAID-22 (c) were presented at the Annual Meeting of the Society of American Bacteriologists, St. Louis, Missouri, May, 1959.

The work has since been greatly extended. It has been found that the nature of the medium in which S. aureus is cultured has a profound influence on its biosynthetic ability to produce penicillinase.

A variety of inhibitors of the induced formation of penicillinase have been examined. Such studies not only offer clues for therapy but also shed light on the nature of protein synthesis.

New methods for the assay of penicillin and related products are being investigated.

The recently developed Nossal bacterial disintegrator has been found to be very effective in the preparation of cell free extracts of S. aureus, an organism which is notoriously resistant to fracture by most conventional procedures.

Some of the results of these studies will be reported on at the Meeting of the Federation of American Societies for Experimental Biology in Chicago, April, 1960, and at the Meeting of the Society of American Bacteriologists in Philadelphia, May, 1960.

Significance:

The continuing studies in carbohydrate biosynthesis are important not only for the selected project dealing with bacterial cell wall material but will contribute to many aspects of carbohydrate metabolism in clinical medicine.

The investigation on the mechanism of penicillin resistance in S. aureus has contributed to the understanding of the nature of protein biosynthesis and has uncovered several leads in the therapy of infections caused by penicillin resistent S. aureus.

Proposed Course of Project:

These studies are being continued.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Huff, E. and Rudney, H.: The Enzymatic Oxidation of 1,2-Propanediol Phosphate to Acetol Phosphate. J. Biol. Chem., 234: 1060-1064, 1959.

2. Huff, E.: Chromatography of Mixtures of Lactaldehyde, Acetol, and Pyruvaldehyde on Bisulfite Ion Exchange Columns. Analytical Chem., 31: 1626-1629, 1959.

3. Huff, E.: Enzymatic Oxidation of 1,2-Propanediol to Lactaldehyde. Federation Proc., 18: 250. 1959.

4. Steinman, H. G. and Murtaugh, P. A.: Isoelectric precipitation of adenovirus and its complement-fixing antigen. Virology, 7: 291-299, 1959.

5. Steinman, H. G. and Murtaugh, P. A.: Nutritional requirements for the induction of penicillinase in Staphylococcus aureus. Bacteriological Proceedings: 101, 1959.

Serial No. NAID - 231-
1. ICI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Systemic fungal disease

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. Albert Treger (to 7/1/59)
Dr. Vincent Andriole (from 7/1/59)

Cooperating Units: Laboratory of Infectious Disease.

Man Years (calendar year 1959):

Total:	1.3
Professional:	1.3
Other:	0

Project Description:

Objectives:

1. To study diagnosis, pathogenesis and natural course of systemic fungus infections.

2. To study the problem of fungus disease due to overgrowth during antibiotic and steroid treatment.

3. To evaluate new agents in antibiotic and chemotherapeutic treatment of systemic fungus infections.

4. To set up means of measuring blood levels of new antifungal agents and to study acute and long term toxic effects of the drugs.

5. To study various immunologic aspects of this group of diseases.

Methods Employed:

Patients with suspected fungal infections are studied diagnostically using the skin test, serologic, histologic and cultural methods. Family contacts are similarly investigated and environmental studies undertaken, when indicated. Infecting fungi from such patients are screened for sensitivity against a considerable number of chemotherapeutic or antibiotic agents and therapy is undertaken after such studies and where clinically advisable.

Drugs are screened for activity against an organism isolated from a patient by *in vitro* methods. Promising drugs are then tested in animals for acute and long term toxicity and for therapeutic effectiveness.

New methods are being developed for determining serum drug levels, rates of excretion of drug, and *in vitro* screening of drugs.

Patient Material and Major Findings:

1. Studies were continued on patients with a variety of fungal diseases. New patients with culturally proved disease this year included cases of cryptococcosis (5), coccidioidomycosis (2), histoplasmosis (5), blastomycosis (3), aspergillosis (2), actinomycosis (2), candida endocarditis or septicemia (2), sporotrichiosis (1).

2. Continued observation of a total of 40 patients treated with amphotericin has permitted more conclusions as to effectiveness. Although clear-cut treatment failures have occurred in 3 cases of cryptococcosis, 4 patients have "apparently recovered" (clinically well with normal cerebrospinal fluid) from their infections 30, 29, 19, and 18 months after cessation of treatment. An additional 5 patients are improved (clinically well but with cerebrospinal fluids not yet normal). Disseminated histoplasmosis has responded well as have patients with blastomycosis, although relapse of infection occurred in 1 patient.

3. Twelve patients with various culturally proved fungal diseases, histoplasmosis (3), blastomycosis (3), coccidioidomycosis (3), candidiasis (1), aspergillosis (1), maduromycosis (1) have been treated with RO-2 (see report of year 1958). This drug in preliminary studies seems effective in some cases of histoplasmosis, blastomycosis, aspergillosis and maduromycosis. Studies are proceeding.

4. An investigation has been undertaken of the side effects of amphotericin (rise in blood urea nitrogen and enzyme) and RO-2 (delay in bromsulphalein excretion) employing biochemic and histopathologic techniques.

5. In a study of histoplasmosis infection in a patient, cultural evidence was obtained of widespread dissemination of the fungus in primary infection. This substantiates the Roentgenographic findings in other patients of splenic calcification following exposure to Histoplasma.

6. The status of therapy of all systemic mycoses was summarized in April 1959 for the American College of Physicians and is to be published.

7. Studies in mice have demonstrated that for diagnostic purposes there exists an optimal time following inoculation of patient specimens for the recovery of Histoplasma and Cryptococcus. This interval for both infections is 2 weeks. Evidence was also obtained that culture of a large volume of patient material (20 ml. of blood, urine or exudate; 5 ml. of cerebrospinal fluid) is an important factor in the isolation of Cryptococcus from patients with meningitis.

Significance to Microbiological Research:

The group of fungus diseases under study is poorly understood, there is no uniformly successful treatment. Apparently, certain of them, such as histoplasmosis, are being recognized as quite prevalent; others such as candidiasis create problems by the very ubiquity of the organism, which may grow more abundantly and cause systemic disease in the presence of most antibiotics and steroids, creating serious and extensive clinical problems.

Proposed Course of Project:

To proceed as outlined under "Objectives" and "Methods."

Part B included Yes [X] No [ ]

Part G: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Utz, J. P., German, J. L., Lourie, D. B., Emmons, C. W. and Carrier, F. L.: Pulmonary aspergillosis with cavitation. N. Eng. J. M. 260: 264-268, 1959.

2. Utz, J. P. and Treger, A.: Current status of chemotherapy of systemic fungal disease. Ann. Int. M. in press.

3. Lourie, D. B., Feder, N., Mitchell, W., and Emmons, C. W.: Influence of fungus strain and lapse of time in experimental histoplasmosis and of volume of inoculum in cryptococcosis upon recovery of the fungi. J. Lab & Clin. M. 53: 311-317, 1959.

* Listed in last year's report as "in press."

Serial No. NIAID - 24(c)
1. LCI
2. Infectious Disease Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Sarcoidosis

Principal Investigators: Dr. Norman H. Bell (to 7/1/59)
Dr. John P. Utz (from 7/1/59)

Other Investigators: None

Cooperating Units: National Heart Institute
National Institute of Arthritis and Metabolic Diseases

Man Years (calendar year 1959):
Total: 0.55
Professional: 0.55
Other: 0

Project Description:

Objectives:

1. Long-term clinical study of the course of sarcoidosis and the effect of hydrocortisone (and related steroid hormones) in altering the manifestations and course of the disease.

2. Clinical studies attempting to demonstrate agents known to produce granulomatous disease in patients with sarcoidosis.

3. Evaluation of the Kveim skin test as a diagnostic tool in the management of sarcoidosis.

4. Study of the calcium metabolic abnormality and the effect of therapy in patients with this manifestation of sarcoidosis.

5. Study of immunological mechanisms in sarcoidosis.

6. Study of the anemia and iron metabolism in sarcoidosis.

7. Study of vitamin D metabolism in sarcoidosis.

8. Epidemiology of sarcoidosis.

Methods Employed:

1. Hospitalization and outpatient observation of patients with sarcoidosis.

2. Extensive cultural and serological study of patients with sarcoid to demonstrate etiological agents.

3. Use of Kveim antigen and a control "antigen" preparation in patients with sarcoidosis and granulomatous diseases of known etiology. Use of pine pollen antigen in both groups of patients.

4. Calcium balance studies on patients with sarcoidosis and hypercalcemia before, during, and after treatment.

5. Calcium infusion, renal phosphorus clearance, and phosphorus deprivation tests before and after treatment with cortisone.

6. Tissue culture of lymph nodes removed from patients with sarcoidosis in an effort to:

   a) study the characteristic aspects of growth of such tissue

   b) study cytotoxic effects of tuberculin and other antigens on such tissue.

7. Fe^{59} iron turnover and Cr^{51} RBC survival studies.

Patient Material and Major Findings:

1. Twenty-five randomly selected sarcoidosis patients with no evidence of blood loss, infection, or hemolysis were studied during the past 2 years. Serial iron determinations over a 24-hour period revealed significantly lower values than those found in controls. Further studies with Cr^{51} and Fe^{59} in 6 patients showed normal red blood cell survival in all but 1, and this patient was 1 of 2 that had rapid plasma iron disappearance. No defect was noted in bone marrow morphology, bone marrow uptake of Fe^{59}, quantitative appearance of iron in red blood cells, or storage of Fe^{59} in liver or spleen. These studies help to define the mechanism of anemia in sarcoidosis and were presented at the meeting of the Southern Section of the American Federation for Clinical Research in New Orleans on January 24, 1959, were published in abstract form, and are being prepared for publication *in toto*.

2. The present knowledge concerning many aspects of sarcoidosis was summarized in a Symposium this year.

3. The clinical and pathologic aspects of sarcoidosis in a mother and daughter were prepared for publication. Only 5 other cases of this disease in parent and child are known, and in 3 of these cases there is some doubt about the diagnosis.

Significance to Microbiological Research:

Sarcoidosis is a disease of unknown etiology which closely resembles granulomatous diseases of known etiology. Investigation of sarcoidosis may provide better understanding of its nature and cause and may shed light on basic immunologic phenomena of tissue allergy. Study of metabolic processes, such as calcium abnormalities, in sarcoidosis provides a basis for more precise characterization of these changes and a rational guide for experimental therapy.

Proposed Course of Project:

To proceed as outlined under "Objectives" and "Methods."

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

1. Utz, J. P., Van Scott, E. J., Bernton, H. W., Edgcomb, J. H., Bunim, J. J., Gill, J. R., Bell, N. H., and Kaufman, H. E.: Sarcoidosis: Combined Clinical Staff Meeting of the National Institutes of Health. Ann. Int. M., December 1959.

2. Merchant, R. K., and Utz, J. P.: Familial sarcoidosis. Accepted by A.M.A. Arch. Int. Med.

Serial No. NIAID - 25(C)
1. LCI
2. Infectious and Pediatric Disease Services
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pyelonephritis

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. Leon G. Smith (to 7/1/59)
Dr. Edward Eyerman (from 7/1/59)

Cooperating Units: None

Man Years (calendar year 1959):
Total: .55
Professional: .55
Other: 0

Project Description:

Objectives:

1. To improve present methods of diagnosing infection.

2. To reproduce the disease in animals to delineate pathogenesis.

3. To evaluate antimicrobial drugs in patients and animals.

Methods Employed:

1. Clinical studies: After standard history, physical examination and laboratory studies, newer laboratory tests will be done and treatment instituted with new drugs.

2. Animal studies: Small animals and monkeys will be used. The effects of bacterial inoculation before and after physiologic or other trauma to the kidneys will be investigated. Serial open biopsies will be done during course of infection. Biopsy material will be studied grossly, microscopically, and culturally. In the case of drugs not used before in patients, toxicity and therapeutic tests will be done on infections in animals.

Patient Material and Major Findings:

1. The need for a convenient method of mass detection of urinary tract infection led to a re-evaluation of the Griess nitrite test. Nitrites are formed in urine as a result of bacterial enzymatic reduction of nitrates and may be detected by the production of a red color on addition of the Griess reagent: a solution containing acetic sulfanilic acid and alphanaphthylamine. Urine specimens were obtained from 336 female job applicants and from patients who had standard urinalyses (3504 specimens) or urinary bacterial cultures (1154 specimens). 97% of patients who had a positive Griess test on urine submitted for urinalysis had a positive culture (> 100,000 colonies/ml.) on a second specimen. Studies of job applicant urines further supported the conclusion that whenever the Griess test was positive there was almost invariably a positive urine culture. However, only 51% of urines with a positive bacterial culture gave a positive Griess test. Negative Griess tests in culturally positive urines appear to be related to several possible factors: inadequate nitrate substrate from food sources, variation in production of nitrate reductase by different species of bacteria, insufficient time for enzymatic action, e.g., in patients with indwelling catheters, and "falsely positive" urine culture. This study has been prepared for publication and is scheduled to be presented at a meeting.

Significance to Microbiological Research:

"Pyelonephritis is the number one problem in infectious disease due to bacteria today." Fully 20% of autopsies show pyelonpphritis, and in a fifth of these cases patients had no history or physical findings during life as warning signs of infection or disease. Furthermore, pyelonephritis is the most common single cause of secondary hypertension. New or simple tests for the presence of infection of the kdineys are urgently needed. The pathologic picture has not been, or is only with great difficulty, induced in animals, and thus stages or factors in pathogenesis are poorly understood. Finally, pyelonephritis is, with the possible exception of staphylococcal infections, the most difficult bacterial disease to treat antimicrobially. Research in treatment must be continued.

Proposed Course of Study:

To be pursued as stated in "Objectives" and "Methods."

Part B included Yes [ ] No [X]

Serial No. NIAID - 2[illegible]
1 LCI
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Individual Project Report
Calendar Year 1959

Part A.

Project Title: Urinary Tract Viruses

Principal Investigator: Dr. John P. Utz

Other Investigators: Dr. David Rifkind (from 7/1/59)

Cooperating Units: None

Man Years (calendar year 1959):
Total: 0.75
Professional: 0.65
Other: 0.1

Project Description:

Objectives:

1. To study the urine of all patients with viral infection for the presence of viruses and to study renal function concurrently.

2. To investigate in animals the effect on the kidneys of the inoculation of virus recovered from human urine.

3. To attempt to produce pyelonephritis in animals by virus inoculation alone or accompanied by secondary physiologic trauma or bacterial infection.

Methods Employed:

Clinical studies: Patients with a variety of viral infection will be studied intensively.

Laboratory studies: Tissue culture and animal inoculation techniques will be used.

Patient Material and Major Findings:

1. Five enteroviruses recovered from urine of patients ill with infection have been studied in animals. Viral content of mouse kidneys at certain times after inoculation is of sufficient titer ($10^{2.5}$ to $10^{4.0}$/mg.) to be poorly if at all explained by viremia. Virus persists in the kidney of the intact animal for a time after virus is last detected in the blood.

2. Coxsackie A-9 virus (not one of types studied in previous experiment) was found in blood urine, kidney and bladders of inoculated suckling mice. Virus was not shown to adsorb to or multiply in mouse kidney or bladder tissue culture explants.

3. No evidence of renal virus infection could be found in rabbits following intravenous inoculation of vaccinia or herpes simplex virus.

4. Studies have been undertaken of the relationship of virus in urine to the presence of inclusion body containing cells in urine.

Significance to Microbiological Research:

The recovery of a number of viruses from the urine of patients raises the question of the role they play in disease. The relationship of this infection to subsequent pyelonephritis needs to be explored.

Proposed Course of Project:

As outlined in "Objectives" and "Methods."

Part B included Yes / / No /X/

SUMMARY

Research Projects from
The Laboratory of Cell Biology
(Serial Nos. NIAID 50-58)

The work of the laboratory is developing along three main lines. The first concerns the metabolism of cultured human and animal cells, described in detail as Project 50. Although progress has been gratifying, two important problems remain to be resolved. The first is the function of the serum protein, which is still required as a supplement to the 28 essential growth factors. Far more important however is the vexing question as to why dispersed cells in culture fail to carry out the specific functions associated with the organ from which those cells were derived. The resolution of this problem has far-reaching implications with respect to mammalian physiology and has a bearing on the problems of antibody production, metabolic disease and cancer. It is hoped that an approach can be undertaken in this laboratory some time during the calendar year 1960.

The second major problem under current study concerns the mechanisms by which cells make viruses, and the effect of viral infection on cellular metabolism. The important advances in this area during the past year by Drs. Darnell, Levintow and Salzman are described in detail in Projects 51 and 52; and we may confidently expect continued productivity in this area. The recent addition of Dr. Wolfgang Joklik as a Visiting Scientist will add materially to the scope of the program.

The third area of investigation has been the use of cell cultures as a tool for the study of human genetics. Although a beginning has been made on the problem of somatic mutation, this program is not represented in this year's report due to the loss of the 2 responsible investigators. Dr. Robert DeMars has joined the Department of Human Genetics at the University of Wisconsin, and Dr. Leonard Herzenberg has joined the staff of Dr. Joshua Lederberg in the Department of Medical Genetics at Stanford Medical School. Dr. Robert S. Krooth, a volunteer worker in the laboratory, has undertaken some important if difficult studies on the cultivation of cells from patients with inherited metabolic disease, in the hope that the biochemical defect will be demonstrable in cell culture, and there amenable to detailed biochemical and genetic analysis. It is contemplated that Dr. Krooth will become a staff member of the laboratory in the summer of 1960.

Recommendations: It is proposed that the activities of the Laboratory of Cell Biology be eventually organized into 3 major sectional sub-divisions: Cellular Physiology, Virus Replication, and Genetics.

The productivity of the laboratory has been materially hampered by lack of space. This is inadequate to the present program and staff, to say nothing of its hoped-for expansion. I believe that we also have an important function as a training center for interested investigators, and that this aspect of our activity can and should be materially increased.

Serial No. NIAID 50
Laboratory of Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biosynthesis in Mammalian Cell Cultures.

Principal Investigator: Dr. Harry Eagle

Other Investigators: Dr. Edward P. Cohen, Mr. Ralph Fleischman, Mr. Aaron Freeman, Miss Mina Levy and Mr. Vance I. Oyama

Cooperating Units: NIDR, Dr. Karl A. Piez
NINDB, Dr. Bernard W. Agranoff
New York University, Dr. Bernard Horecker
University of California, Dr. E. E. Snell

Man Years (calendar year 1959):
Total: 10-3/4
Professional: 6
Other: 4-3/4

Project Description:

Objectives: This program involves a broad exploration of the nutritional requirements, metabolic activities and biosynthetic pathways of a variety of human and animal cells, under varying conditions of culture.

Methods Employed: The techniques used in the culture and chemical fractionation of these cells, in the separation and identification of their metabolic products, and in the use of isotopically-labeled precursors, have been described in detail in previous publications.

Major Findings:

a. Amino acid biosynthesis and interconversion. Of the 7 nutritionally essential amino acids three (alanine, serine, glycine) derive 70-80% of their carbon skeleton from glucose and 2-10% from glutamine. Four others (aspartic acid, asparagine, glutamic acid proline) derive 70-80% of their carbon from glutamine and 2-10% from glucose. Only 80-90% of the carbon chain is thus accounted for; and the source of the remaining carbon is under present study. Particular interest attaches to the fact that ribose and pyruvate

Part B included.

together substitute for glucose in the growth of a variety of human cell cultures. The degree to which these two compounds are used in amino acid biosynthesis is under current study.

Although mammalian cells contain a wide spectrum of transaminases, current studies with N^{15}-labeled amino acids have shown that only one of those transaminases is of physiologic significance in relation to the provision of the α-NH_2 group. The 7 nutritionally non-essential amino acids which the cell synthesizes in a minimal medium derive essentially all their amino nitrogen from glutamic acid. Free NH_3 is essentially inactive.

It had previously been shown that glutamic acid would substitute for glutamine only at high and non-physiological concentrations. Dr. Robert DeMars has shown that at those high concentrations the cells adaptatively form increased amounts of glutamine synthase. Having formed the enzyme, the cells can then grow at low levels of glutamic acid. This is the first reported instance of adaptive enzyme formation in mammalian cell cultures.

Cystine is a rigorously essential amino acid. Despite this fact, the cells have a significant capacity to synthesize cystine, using methionine as the S source and glucose (serine) as the C source. This is one of a number of instances in which the cells have an absolute requirement for a growth factor despite a significant capacity for its biosynthesis.

b. The utilization of serum protein. Experiments with C^{14}-labeled proteins have shown that the proteins of the cell are derived almost in their entirety from the free amino acids of the medium, and that the serum protein in the medium accounts for less than 3-6% of the cell protein. Because of the several opportunities for contamination of the cell protein with medium (mechanical adhesion, pinocytosis, bound amino acids) the 3-6% is a maximum figure, and the true value may be as little as 1% or less.

The apparently contradictory observation that serum protein may serve as a source of cystine in an otherwise cystine-free medium has been shown to be due, not to the breakdown and utilization of the cell protein itself, but to the fact serum proteins, by virtue of a free-SH group, bind cysteine in a sulfhydryl-disulfide interchange reaction. This tightly bound cysteine resists dialysis and even treatment with trichloracetic acid; but it can be mobilized in cell culture by any reduced sulfur compound, and thus become available to the cell for growth. When the protein is depleted of this bound cysteine it becomes inert as a substitute for cystine.

c. Amino acid, glucose, and PO_4 transport. Data are gradually accumulating with respect to the degree to which amino acids

are concentrated by cells, the rate of that transport process, and the degree of variation among individual cell strains. In collaboration with Dr. Bernard Horecker of New York University, studies are similarly in progress with respect to the transport of glucose, phosphate and glucose-6-phosphate. Surprisingly, preliminary evidence indicates that glucose-6-phosphate can be transported into the cell as such without its preliminary hydrolysis to glucose.

d. Nucleic acid biosynthesis. Previous experiments have shown that the pyrimidines derive approximately half of their carbon from glutamine, and that approximately 50% of the total nucleic acid base nitrogen derives specifically from glutamine amide nitrogen, rather than ammonia. The pathways involved in the biosynthesis of ribose and deoxyribose are currently under study.

e. Protein synthesis and protein turnover. Preliminary experiments indicate that the rate of protein synthesis is determined by the concentration of the free amino acids in the cellular pools. There is no measurable synthesis until the concentration of each amino acid exceeds a critical threshold level, on the order of at least 0.01 mM; and the rate of protein synthesis is essentially maximal at 0.05-0.1 mM. These data are consistent with the thesis that protein synthesis involves the simultaneous assembly of a large number of amino acid residues on a template, rather than their sequential addition.

It is generally considered that protein turnover involves the continuing breakdown of the cellular protein to amino acids followed by their re-assembly. Recent data, however, indicate that a number of metabolic inhibitors which completely block net protein synthesis have no demonstrable effect on the rate of protein turnover. The implication is clear that the replacement of cellular proteins occurs by some process qualitatively dissimilar from that involved in the synthesis of new protein.

f. Inositol biosynthesis. It had previously been found that 21 of 22 cell strains examined had a rigorous requirement for myo-inositol. In collaboration with Drs. Agranoff and Snell it has been shown that these cell lines requiring inositol nevertheless have a significant ability to synthesize this growth factor from the glucose of the medium. This is another example of a rigorous requirement by cells for a growth factor in the face of a significant capacity for its biosynthesis. The one cell line which did not require inositol was found to release it into the medium at concentrations 10^{-6} gm/ml. This suggested the possibility of a parabiosis experiment, in which the cell strain which produces inositol would feed an inositol-dependent strain. This has in fact been accomplished.

g. Morphologic changes in mammalian cells grown in an amino acid deficient medium. The purpose of this project is to identify by electron microscopy the early morphologic changes when cells are placed in an amino acid deficient medium. When HeLa cells were placed in a valine-deficient medium, minor changes in the integrity of the nuclear membrane and gross mitochondrial swelling developed within four hours. After 12 hours, there was obvious disruption of the nuclear membrane.

h. A simple chemostat for the growth of mammalian cells. A simple chemostat has been developed for the growth of mammalian cells in a controlled and constant environment. The apparatus consists essentially of 3 glass vessels in series, one containing a reservoir of fresh medium, one the growing culture, and one for the overflow. The inflow of medium is controlled either by a constant-head gravity pump, or by a Sigma peristaltic pump; and the middle cell reservoir is maintained at constant volume by an overflow leveling tube. For each volume of fresh medium which enters the cell culture, an equal volume of spent medium and cells overflows into the third reservoir. No other control is required, in that the rate of cell growth and the population density are controlled by the rate of inflow of new medium. Under conditions of optimum growth, the cells divide approximately every twelve hours. Qualitatively, the system follows the kinetics of growth as determined for bacterial growth under similar conditions.

The growth of mammalian cells in the chemostat provides an opportunity for the study of various parameters of cellular metabolism under conditions of both rapid and slow growth.

Significance to the Program of the Institute: Studies on the metabolic activities of human and animal cells are of obvious basic significance. For NIAID research specifically, these studies are relevant to the problems of antibody production, allergy, phagocytosis, inflammation, virus propagation, and many other problems which are the concern of the microbiologist interested in the study of human disease.

Proposed Course of Program: It is clear from the foregoing that none of these are finished projects. The long-range program envisages a similar broad-scale study on the metabolism of tissue explants. This will have the dual purpose of pin-pointing possible differences between the metabolism of dispersed and structurally organized cells, and exploring the conditions necessary for the preservation of specialized functions in vitro.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Lockart, R.Z., Jr. and Eagle, H. Requirements for Growth of Single Human Cells. Science 129: 252-254, No. 3344, 1959.

Eagle, H., Piez, K.A., Fleischman, R. and Oyama, V.I. Protein Turnover in Mammalian Cell Cultures. J. Biol. Chem., 234: 592-597, 1959.

Darnell, J.E., Jr., Eagle, H. and Sawyer, T.K. The Effect of Cell Population Density on the Amino Acid Requirements for Poliovirus Synthesis in HeLa Cells. J. Exptl. Med. 110: 445-450, 1959.

Eagle, H. Amino Acid Metabolism in Mammalian Cell Cultures. Science 130: 432-437, 1959.

Eagle, H. and Piez, K.A. The Utilization of Proteins by Cultured Human Cells. J. Biol. Chem. in press.

Eagle, H., Oyama, V.I. and Piez, K.A. The Reversible Binding of Cyst(e)ine by Serum Protein, and its Bearing on the Cyst(e)ine Requirement of Cultured Mammalian Cells. J. Biol. Chem. in press.

Levintow, L. and Eagle, H. The Technique of Mammalian Cell Culture. Methods of Enzymology, Vol. 5, in press.

Honors and Awards relating to this project:

Lectures

February 18, 1959: Research Seminar, Charles Pfizer & Company, Brooklyn, N.Y.

February 19, 1959: Harvey Lecture, New York City

March 20, 1959: Sigma Xi Lecture, University of North Carolina, Chapel Hill, North Carolina

April 12-18, 1959: The Metabolism of Mammalian Cells, Symposium on Cell Cultures, Inter-society meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N.J.

May 13-16, 1959: Ciba Symposium, guest speaker, Symposium on Human

Serial No. NIAID 51
Laboratory of Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Mechanism of Polio Virus Production in Cultured Mammalian Cells.

Principal Investigator: Dr. James E. Darnell, Jr.

Other Investigators: Dr. Leon Levintow, Mr. J. Leonard Hooper, and Miss Marilyn Mitchell.

Cooperating Units: None

Man Years (calendar year 1959)
Total: 5-3/4
Professional: 2
Other: 3-3/4

Project Description:

Objectives: To define in biochemical terms the adsorption of virus to a susceptible mammalian cell, and the replication and maturation of the virus within the host cell.

Methods Employed: The work has been concerned up to now with the poliovirus-HeLa cell system, and has depended principally on the following basic techniques:

a) A sensitive plaque assay for the quantitative estimation of poliovirus (Darnell, J. Exp. Med. 107, 633, 1958).

b) The infection of cells under controlled conditions and,

c) The purification and characterization of poliovirus from such infected cultures (Levintow and Darnell, J. Biol. Chem. in press).

Other important techniques which have been employed include immunological procedures for the quantitative measurement of viral protein, paper and column chromatography, preparative ultracentrifugal fractionation by the equilibrium gradient technique, and various microanalytical chemical procedures.

Major Findings: The variation in susceptibility to poliovirus among various clonal strains of HeLa cells has been found to be due

Part B Included

neither to a failure of adsorption of virus to the resistant cells, nor to their resistance to viral RNA. These findings lead to the conclusion that the viral RNA is made available with relative inefficiency in the resistant cell.

Previous nutritional studies had suggested that poliovirus protein is assembled from the large amino acid pool of the infected cell. This suggestion has been directly confirmed by experiments using cells differentially labelled in protein and in the pool with C^{14}-valine. Virtually none of the cell protein proved to be utilized for virus synthesis.

The time at which the infected cell synthesizes the virus-specific protein and ribonucleic acid has been determined by using radioactive precursors of the protein and of RNA. Although the synthesis of both virus RNA and protein begins at about the same time as mature virus begins to appear, the protein is completely formed 1-2 hours before maturation is complete. The exact course of RNA synthesis has not as yet been accurately determined

Significance to the Program of the Institute: Virus replication provides a useful model of a rapidly reproducing unit level at the cellular and subcellular/in which both the nucleic acid and protein components can be studied chemically and biologically. The practical implications of such studies with respect to the evolution of viral disease requires no elaboration.

Proposed Course of Project: The availability of a simple method for purification of poliovirus permits a wide variety of experiments including:

1. Attempts to study the fate of the infecting virus particle.

2. Experiments involving inhibitors of virus protein synthesis aimed at determining whether virus protein and nucleic acid may be independently synthesized.

3. Detailed study of poliovirus protein.

4. The differences in chemical composition of various strains of virus.

5. Production of heavy atom (Hg) derivatives of poliovirus for crystallographic study.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Variation in Plaque-Forming Ability Among Parental and Clonal strains of HeLa Cells. Virology 8: 223, 1959. J.E. Darnell, Jr., and T. K. Sawyer.

The Effect of Cell Population Density on the Amino Acid Requirements for Poliovirus Synthesis in HeLa Cells. J. Exp. Med. 110: 445, 1959. J.E. Darnell, Jr., Harry Eagle, and T. K. Sawyer.

A Simplified Procedure for Purification of Large Amounts of Poliovirus: Characterization and Amino Acid Analysis of Type I Poliovirus. J. Biol. Chem. in press. Leon Levintow and J.E. Darnell, Jr.

Poliovirus Protein: Source of Amino Acids and Time Course of Synthesis. J. Biol. Chem. in press. J.E. Darnell, Jr. and Leon Levintow.

Honors and Awards relating to this project: None

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FY5-FY6
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effects of Animal Viruses on the Metabolism of Mammalian Cell Cultures and the Mechanism of Viral Replication.

Principle Investigator: Dr. Norman P. Salzman

Other Investigators: Mr. Edwin D. Sebring and Dr. Royce F. Lockart, Jr.

Man Years (calendar year 1959):
Local: 1 1/2
Professional: 2
Other: 1 1/2

Project Description:

Objectives: To determine the alterations in cell metabolism resulting from infection with poliovirus and vaccinia virus, and to study the primary site of action of the virus; to determine the time course of synthesis of the component parts of vaccinia virus (nucleic acid and protein) as related to the formation of infectious virus, and to study the effect of inhibitors on these synthetic processes.

Methods Employed: The techniques employed involve cell fractionation, isolation of the purified components nucleic acid bases, the use of radioactive precursors, the various techniques required for the viral infection of cells, the plaque assay for measurement of infectious virus, and chemical methods of virus purification.

Major Findings: Our previous studies on the effect of poliovirus infection on HeLa cell metabolism established that DNA synthesis was inhibited in infected as compared with control cultures, but low levels of synthesis were observed. A metabolic inhibitor (5-fluorodeoxyuridine, or FUDR) which specifically blocked DNA synthesis, but allowed RNA and protein synthesis to continue, completely blocked the formation of vaccinia virus, but had no effect on poliovirus formation. This established that

Part B included.

de novo DNA synthesis is not required for the formation of an RNA virus. These studies suggest also that FUDR may be generally useful to determine if a virus of unknown chemical constitution contains RNA or DNA. The rate of vaccinia DNA formation has been determined in infected HeLa cells by adding FUDR at intervals after infection. Viral DNA formation is almost complete prior to the formation of mature infectious virus.

It had been observed that poliovirus results in a breakdown of cellular RNA. By the examination of various cell fractions and of the purified virus, it could be shown that these breakdown products were not utilized for viral synthesis. Instead, poliovirus ribonucleic acid was derived from the pre-existing small molecular weight material in the cell pool.

Significance to the Program of the Institute: The effect of viruses on animal cells, the manner in which viruses replicate, and the effect of inhibitors on these processes is of obvious relevance to an understanding of viral infection, and may conceivably suggest new areas of exploration in relation to viral chemotherapy.

Proposed Course of Project:

1. Current studies are designed to determine whether viral infection is in itself lethal to a cell, or whether cellular death is a result of viral replication.

2. The effect of a DNA virus, vaccinia, on cellular metabolism will be studied in comparison with that of an RNA virus-poliovirus.

3. The effect of metabolic inhibitors on viral replication will be studied.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Salzman, N.P. Systematic Fluctuations in RNA, DNA and Protein During Growth of Mammalian Cell Cultures. Biochim. Biophys. Acta, 31: 158 (1959).

Salzman, N.P., and Lockart, R.Z., Jr. Alteration in Ribonucleic Acid Metabolism Resulting from Poliomyelitis Virus Infection of HeLa Cells. Biochim. Biophys. Acta, 32: 572 (1959).

Salzman, N.P., and Sebring, E.D. Utilization of Precursors for Nucleic Acid Synthesis by Human Cell Cultures. Arch. Biochem. Biophys., 84: 143 (1959).

Salzman, N.P., Lockart, R.Z., Jr., and Sebring, E.D. Alterations in HeLa Cell Metabolism Resulting from Poliovirus Infection. Virology, 9: 244 (1959).

Salzman, N.P. The Rate of Formation of Vaccinia Deoxyribose Nucleic Acid and Vaccinia Virus. Virology. In press.

Honors and Awards relating to this project: None.

Section Unit NIAMD
Laboratory of Cell Biology
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Carbohydrate Metabolism in Cell Culture.

Principal Investigator: Dr. Stanley Barban

Other Investigators: Mr. Henry O. Schulze

Cooperating Units: None

Man Years (calendar year 1959)
Total: 3
Professional: 1
Other: 2

Project Description:

Objectives: To study the effect of a metabolic inhibitor, deoxyglucose, on the carbohydrate metabolism of human cells in culture.

Methods Employed: The techniques employed in the culture of these cells, in the separation and identification of the amino acids, carbohydrate intermediates, and their metabolic products, and the use of isotopically labeled precursors, have been described in detail in previous publications relating to this project.

Major Findings: The glucose analogue, 2-deoxy-D-glucose, has been found to inhibit the growth of cultured mammalian cells, with no demonstrable differences between cells deriving from normal and malignant tissues. The degree of inhibition depended on the molar ratio of carbohydrate to inhibitor, and was related also to the carbohydrate substrate. At equimolar concentrations of glucose (or mannose) and 2-deoxy-glucose, there was almost complete inhibition of growth. Cells grown on fructose were more sensitive to the inhibitory effect of 2-deoxy-glucose. The competitive nature of the growth inhibition was demonstrated by

Part B Included

reversal with added substrate. 2-deoxy-glucose has been shown to inhibit glucose transport in these cells.

Enzymatic studies have shown that 2-deoxy-glucose is readily phosphorylated to 2-deoxy-glucose-6 phosphate by cell-free extracts of cultured cells. Unlike the parent 2-deoxy-glucose, this phosphorylated compound inhibited the action of phospho-glucose isomerase and glucose-6-phosphate dehydrogenase.

Significance to the Program of the Institute: An understanding of the effects of an inhibitor of carbohydrate utilization is of importance in elucidating the pathways of cellular metabolism. Such studies are an essential preliminary to the exploration of the biochemical changes associated with disease.

Proposed Course of Project:

Experiments are in progress to define the precise metabolic step at which the glucose analogue affects the carbohydrate metabolism, and thus, the growth of cultured cells.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Transamination Reactions of Mammalian Cells in Tissue Culture.
Barban, S., and Schulze, H. O. J. Biol. Chem., 234: 829-31, 1959.

Honors and Awards relating to this project: None

Preface to Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1959

You will note that my summary, in addition to describing research aims and accomplishments includes, in an unprecedented manner perhaps, certain comments concerning the adequacy of space, personnel and finances in LID. I find it increasingly difficult in this day of modern research to divorce such questions as research goals and accomplishments from the equally vital questions concerning the resources available for achieving them. If the latter are less than adequate for this purpose, then this becomes a most critical and appropriate subject for any summary and appraisal of program.

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Summary Statement
Laboratory of Infectious Diseases, NIAID
Calendar Year 1959

Introduction

During 1959 the Laboratory of Infectious Diseases continued as its primary interest, investigations of the roles viruses, bacteria and fungi as causes of disease. Because of their greater competence to do so, I have asked the heads of the sections on Mycology and Bacterial Physiology to summarize and discuss those research programs (vide supra).

Virus and Epidemiology Sections

Since the research program of the viral and epidemiological sections are almost wholly integrated, I have described the aims and the work of these sections together. Their closely interwoven research programs are designed to define the behavior and importance of viruses (respiratory viruses, enteroviruses, and cancer viruses) in their natural hosts, observed in certain natural environments. Thus, studies of respiratory diseases and other undifferentiated illness were continued in two pediatric groups - at Junior Village and at Children's Hospital, and in groups of adults, including several installations of the U S. Navy and at the University of Maryland The role of viruses in respiratory and intestinal diseases of cattle were observed on farms, feedlots and in experimental pens at the Universities of Maryland and Illinois. The potencies and possible effectiveness of respiratory virus vaccines were examined in a preliminary manner in relation to both human and animal respiratory illness, and broad seroepidemiologic studies of the prevalence of many viruses in various age and economic groups in various parts of the world were initiated.

During 1959 viruses as a cause of tumors and cancer in animals developed into the major research interest of nearly one-half the personnel of the virus section. At first our studies were focused on the development and evaluation of new techniques for demonstrating and detecting tumor viruses in experimental mouse colonies and chicken flocks. In July, 1959 we turned our attention to the occurrence of mouse and avian tumor viruses in natural populations.

This research program requires close but freely given collaboration not only between the various units in the two sections of LID, but also with a number of other research groups, both within and outside the NIAID*.

*D.C. Welfare Department; Dairy Husbandry Dept., College of Agriculture, Univ. of Maryland; Maryland Home of Correction; University of Maryland, Student Health Service (Drs. Dyke and Iler); Bureau of Med. and Naval Med Res Institute (Drs. Kingston, Bloom, Mufson and Gordon); Children's Hospital Res Found. (Dr. Parrott); NINDB Collaborative Study (Dr. Masland); National Cancer Institute (Drs. Kahler and Law).

Our main objective could be described as a comprehensive understanding of the virus experiences of man and certain animals, particularly, of course, of those that the evidence indicates are the most important. All available laboratory tools and epidemiologic methods (some developed in LID) are employed to whatever extent may be necessary to achieve this end. This is a large operation in pursuit of large objectives made possible by the exceptionally large and sympathetic support given by NIAID and the National Institutes of Health. The success of the operation depends on teamwork and on long term planning of a unique order which permits maximum freedom and flexibility to the cooperating units, yet maintains a steady progression towards synthesis of data and interpretations designed eventually to provide a comprehensive picture of the extremely complex, ill-defined problem produced by more than 100 common human viruses.

Unfortunately our effort promises to become even more unique, since in most academic circles current trends in virus research are away from studies of naturally occurring disease, favoring instead studies of laboratory models, apparently on the assumption that natural viral phenomena are now too complex (and therefore too expensive) to be properly studied within the framework of contemporary information and thinking about what can be done in university environments.

Viral research and teaching programs are almost wholly concerned with laboratory models aimed at understanding the chemical structure and behavior of viruses (not more than several) as observed in an ultra-microcosm - namely, cells in tissue culture; all of which are worthy objectives with tremendous possibilities. This development in itself would not be disturbing were it not for the likelihood that it will seriously reduce the supply of medical virologists with broad training and interest in virus as a cause of disease; and this at a time when the opportunities have never been better for solving some of man's knottiest and most frequent disease problems

We feel that the complex but interlocking virus disease problems in nature cannot yield to the assaults of the solitary observations of the lone virus worker. We feel it is one of the functions of a research group to do what we can to enlarge current horizons, to show what can still be discovered in nature - the "real universe".

It is our contention that the most important thing to know about a laboratory "model" is not how beautiful, elegant, or precise it may be, but whether or not it really is a "model" of something which exists in nature, and that the most essential information about a virus is what it actually does, not what it is made of or what it might do in an artificial system. Both these links with reality can only be achieved by observing natural behavior in nature. (For further comments on "Trends" see "Perspectives in Respiratory Disease Research" and "Some Questions about Possible Approaches to Viruses as a Cause of Cancer".

Respiratory Virus and Enterovirus Studies

The National Health Survey 1957-1958 revealed once again and with greater force than ever the magnitude of Acute Respiratory Diseases in the U.S.A. It was estimated that several hundred millions such illnesses had occurred and confirmed previous impressions developed in several independent studies, including those in LID, about their greater prevalence in children. Consequently, studies of this essentially unsolved problem were pursued with undiminished vigor by the various sections and units in LID. Although our major efforts were still aimed at further definition of childhood respiratory viral disease, somewhat more effort went into studies of the importance of certain viruses in adult respiratory disease and into preliminary evaluations of commercially prepared respiratory vaccines.

The Junior Village orphanage study under Dr. Bell's general supervision, now in its 5th year, continued to serve magnificently in its dual role as (1) a source of basic and essentially new information on the total viral and bacterial experiences of the urban infant, and (2) serving as a focal point for a number of more specific studies on the behavior and possible prevention of certain of the apparently more important agents.

Analysis of the overall experiences, now completed, for 3 years continued to show that an average of 25% of the infants suffered a new acute febrile illness (exclusive of measles and chickenpox) each week; and that approximately 30% of them acquired a new identifiable viral or bacterial infection during the same interval. A total of over 1933 separate infections with viruses belonging to 53 different serotypes and 718 infections with presumed bacterial pathogens were observed. Crude estimates indicate that 40% to 45% of the more severe illnesses observed in the orphanage infants could be attributed to known agents, most of them viral.

Dr. Bell's statistical analysis showed certain agents were significantly and repeatedly associated with febrile illnesses. These included adenoviruses types 1, 3, and 5, influenza A, parainfluenza 1 and 3, poliovirus 2, Coxsackie B5, Group A streptococci 4, 12, 23, Shigella sonnei, and, of course, measles and chickenpox viruses. Others, including many respiratory and enteric viruses and bacteria presumed to cause illness, failed to produce sufficient illnesses in excess of the high "normal" rates to provide significant associations. Although these latter observations do not exclude the likelihood that under other circumstances they might well produce clearcut illnesses, repetitive occurrences of viruses and bacteria over several years in this carefully studied population provide valuable information on the relative pathogenicities of many common agents. As these and supplementary studies in Children's Hospital continue, we should find it possible to ascertain the changes in patterns of microbial experience and herd immunities which take place during the passage of time. Very important theoretical questions about the importance of viral mutation and perhaps recombination have never been studied effectively in relation to natural human infection and disease. Our background information on Junior Village without doubt provides the first good opportunity to finally develop real information on the actual validity of these concepts concerning the evolution of infectious agents and the diseases they cause.

Delineation of Specific Viral Diseases

Perhaps of even more importance at this time are the specific and original contributions to our growing fund of information about the existence and behavior of new human viruses, first discovered or delineated in our Junior Village studies.

Rowe and Hartley found Junior Village valuable in developing much new information on the natural occurrance of salivary gland virus, particularly its continuous presence in the oral secretions of many infants. Chanock, Cook, and others discovered the first hemadsorption viruses (parainfluenza 1 and 3) in Junior Village specimens, and the first outbreak defining the clinical importance of parainfluenza 3 was reported in this population. In 1959 three large outbreaks of parainfluenza 3 showed that although reinfection of infants with serum antibodies occurred, increasing levels of antibody provided increasing protection against both infection and illness. Ten percent of 120 cases had pneumonia - all of them occurred in infants without antibodies or other evidence of previous infection. Recently Johnson was able to confirm his discovery of a fourth parainfluenza virus during an outbreak of infection with this virus in Junior Village. Bell, Rowe, and Huebner, using the Junior Village study system, finally developed conclusive evidence that most of the adenoviruses that commonly infect children also cause significant amounts of illness - nearly 50% of the infections with types 1, 3, and 5 showing clearcut evidence of association with febrile illness. Similarly, Rosen discovered a number of new serological prototypes of adenoviruses, including a group of them that can be demonstrated only in the intestinal contents. He employed to good purpose the numerous readily available adenoviral infections in Junior Village to develop a new and enormously valuable HI technique for specific detection of infection with specific adenoviruses. The natural occurrances of ECHO 10-like viruses observed in this population provided the necessary information employed in 1959 by Rosen and Sabin to construct the 3 separate prototypes, the new reovirus group. The new HI techniques used for diagnosis of infections with these agents were also developed by Rosen using serums from patients in Junior Village with demonstrated reovirus infections. This procedure was used to determine much of what is known about the epidemiology of these agents.

The prototypes of several newly characterized ECHO viruses were first discovered by Rosen in Junior Village and the only information available on the epidemiology of many of the ECHO viruses was also acquired in Junior Village.

In 1959 Dr. Dalldorf and his associates initiated preliminary controlled tests of commercially produced experimental respiratory virus vaccines in Junior Village. Adenovirus, influenza, Coxsackie B, parainfluenza and measles vaccines, all of them inactivated by formalin, were placed under study in the orphanage. Recently a multivalent vaccine, including 12 different antigens, was also introduced. Except for suggestive evidence of some protection from measles vaccine, the Junior Village vaccine studies have served chiefly to re-emphasize that antibody response of young infants to inactivated viral antigens is remarkably less than that of adults and that inactivated vaccines of much greater antigenic potency will be required to support any reasonable hopes for producing adequate protection in young children. The achievement of this aim will require intensive future studies on concentration and purification of viral antigens, and on adjuvants. Studies of this type are best suited to industrial research programs and we are inclined to leave this to them - consulting of course with their scientific staffs concerning desirable components and potency levels indicated by our epidemiological data and study opportunities.

Several of our virologists have developed considerable interest in the possibilities of attenuated live vaccines for prophylaxis of common respiratory virus illnesses, and currently Johnson and Chanock are planning to select variants of certain viruses by adapting them to grow at different temperatures. One rather convincing school of thought holds that effective eradication of common virus diseases may someday be achieved through universal long term immunization with live attenuated agents. Much, however, needs to be learned about the possible cumulative late effects of numerous viral infections that are clinically inapparent (including those induced by attenuated viruses) in their natural hosts. This can only be achieved by much more comprehensive epidemiological studies of naturally occurring infections than anyone now contemplates. There is considerable question (based on studies of interference phenomena and "interferon") whether or not it will be a good thing to eliminate large numbers of common but non-fatal virus infections. Such questions can only be answered by much more data indeed.

Seroepidemiologic Studies

The development and evaluation of suitable methods for acquiring large amounts of data on viral infections in large general and selected populations represents perhaps our major current activity in the field of viral technology. Thus we and others have now developed complement fixation and HI procedures for measuring type specific serum antibodies to more than 40 viruses and with the use of group specific antigens it is now technically possible to test for antibody responses to 75 or 80 viruses

We think this represents a major breakthrough which can now be exploited to carry out really comprehensive seroepidemiological studies of human viral experiences in any special or general frame of reference. Thus differences between the prevalence of many agents can now be determined for different times, different geographic areas, different economic or social groups, and different ages. The occurrence of common viral infections of both wild and domestic animals and the likelihood of some of these being shared between species, can now be studied. Well controlled seroepidemiologic studies will be of particular value in developing leads concerning needed viral vaccines and the roles of viruses in neonatal, chronic, and neoplastic diseases.

Our seroepidemiologic studies were much advanced in 1959 through the collaborative study undertaken with NINDB, particularly through a contract with Microbiological Associates, Inc., which has successfully developed and produced in large amounts the majority of viral antigens needed. Five of 7 technical positions allocated by NINDB to the Virus Section were satisfactorily filled, and the technicians have been carefully trained in the performance of various serological procedures, and in the simultaneous use of numerous viral antigens.

Our plans for the future call for gradual expansion in scope and magnitude of seroepidemiologic studies (we are now effectively using only 15 viral antigens in our routine diagnostic studies). During fiscal year 1960 the first broad tests should be completed on serial specimens from the NINDB collaborative study, on birth defects, cerebral palsy, mental retardation, and sensory disorders of infancy. Global seroepidemiology of numerous common viral infections now also appears entirely feasible and could be of enormous importance in increasing understanding of the varying patterns of virus infection and disease in all parts of the earth; such information, if extensive enough, conceivably might allow us eventually to predict major viral epidemics.

Specific Respiratory Disease Studies

The role of the "Eaton" agent in Primary Atypical Pneumonia (PAP) has been a controversial one for many years. Furthermore, to those who accepted it as one of the causes of nonbacterial pneumonia the importance of the agent as a cause of disease and its natural history remained unknown. Drs. Chanock and Cook and others in LID with the Bureau of Medicine and Surgery, USN, studied longitudinally continuous endemic occurrences of primary atypical pneumonia at a Marine recruit training center in the South. The Coons fluorescent antibody technique adapted in LID for large scale routine diagnosis of PAP made the study possible. Approximately 70% of all pneumonia observed was associated with Eaton virus infection, whereas 3% of persons with febrile respiratory illness and 6% of persons with no respiratory illness showed rises to PAP. The ratio of illness to infection was approximately 1 in 12. The existence of this rather large clinically inapparent segment of PAP was unexpected. The data obtained from military recruits was supplemented by information on the occurrence of PAP in children observed in the cooperative study at Children's Hospital. Here it was found over a 16 month period that 10% of lower respiratory illnesses was associated with infection with the Eaton agent. More recently, working with Dr. Francis Gordon and his associates, Drs. Cook and Chanock succeeded in growing the PAP agent in this way, which appears to be an important breakthrough

Comment

Recent analysis of Health Statistics by the American Statistical Society shows a gradual increase in the incidence of pneumonia and in deaths due to pneumonia over the last several years - an increase which appears quite independent of the impact of Asian influenza. The fact that we now recognize several viral causes of pneumonia in addition to influenza, namely, adenoviruses (types 1, 3 and 7), parainfluenzas 1 and 3, the Eaton agent, and even measles virus, increases the urgency for renewed and much greater research efforts to determine more specifically the viruses which cause nonbacterial pneumonias.

Parainfluenza 4

During a study of minor respiratory illnesses in university students Dr. Karl Johnson isolated a strain of virus in tissue culture (M-25) which proved very hard to handle in the laboratory. However, after nearly a year of study the agent was shown to be a hemadsorption virus - distinct from parainfluenza types 1, 2, and 3. It was found occurring in Junior Village. Like the respiratory syncytial virus, M-25 appears, from serological surveys, to be very prevalent; however, because of difficulties in isolating the virus in suitable epidemiological studies capable of determining precise association with illness, and therefore its medical importance, is as yet unknown.

Studies of Enteroviruses

Dr. Leon Rosen left for 6 months duty in Tahiti in August, 1959. The Laboratory Unit of the Epidemiological Section continued its survey of enteroviral experiences in Junior Village. Before leaving, he completed interesting laboratory and epidemiological observations on ECHO 10 viruses. Using a type specific HI test he delineated 3 distinct serotypes amongst the assorted strains isolated from Junior Village. This information provided much of the basis for the new viral genus called reoviruses by Sabin. Distinct moderate outbreaks of types 1 and 3 have occurred in Junior Village; however, so far there is only borderline evidence suggesting that these viruses cause febrile illness. Rosen's studies in Tahiti have lead to the demonstration of indigenous infections with all 3 reoviruses, certain ECHO viruses and adenoviruses. ECHO 14, and adenoviruses types 1, 2, and 16, have been isolated. A recent sharp outbreak of respiratory disease was shown to be due to influenza B.

The [illegible] by [illegible] and Schnurrenberger that parainfluenza virus [illegible] is isolated from young cattle [illegible] shipping fever [illegible] to further studies [illegible] virology of [illegible] viruses in the bovine species. Subsequently, in the University of [illegible] Division of Veterinary Science, and the College of [illegible] School of Veterinary Medicine, Dr. Reinhart found parainfluenza 3 to be one of the most common virus infections of cattle - often producing little or no clinical disease. As in man, reinfection with the virus was demonstrated. Despite occasional [illegible] produced in calves by artificial inoculation and the recovery of the agent from pneumonic lungs the role of the virus in producing shipping fever was not determined. Commercial vaccine, with and without adjuvants produced antibody levels comparable to modifying the virus propagation in subsequent challenge. Contact [illegible] studies designed to determine the ability of the vaccine to protect against shipping fever were vitiated by the failure of shipping fever to occur even in controls in the one study that could be completed. Insufficient [illegible] precluded more definitive subsequent studies.

Longitudinal laboratory and epidemiological studies of cattle populations have yielded many additional viruses including infectious bovine [illegible] tracheitis, sunery orthomyxoviruses, and all 3 reoviruses. IBR virus, which resembles adenovirus in its physical and biological properties was shown to have caused an extensive outbreak of catarrhal conjunctivitis in cattle [illegible] in Eastern Maryland.

Comment

The field aspect of these studies are carried by Dr. Reinhart [illegible] only by such help as is furnished by cooperating institutions. The laboratory work - virus isolation and serology - are done largely by one of the most experienced technicians Carlton as one of the WHO positions, consequently she will not be available very long to assist in this interesting and important research project. This program is unique - and could be very useful in providing indispensable information on [illegible] probably now on the rise for one of the common human virus infections - and who [illegible] and deserves support. Recent investigations by Dr. Reinhart on [illegible] and a virus causing virus have confirmed both of these as [illegible] [illegible] moreover, serological evidence of positive human infections were also found. Thus, there are several large groups of viruses (3 or 4 [illegible] viruses) which may be shared by man and domestic animals.

A few years ago there was much general [illegible] about the [illegible] of swine influenza which [illegible] in [illegible] years of [illegible] interest. It appears that there are [illegible] relationships of [illegible] [illegible] infections and enteroviruses also [illegible] occurring in both man and swine. One could create a great deal of excitement in the general [illegible] over such interspecies [illegible] in [illegible] to study such problems as [illegible] of the [illegible] virus, but we can't do it [illegible] with [illegible] personnel and [illegible] facilities.

pursuit of [illegible] progress. Tumor virus research and virus [illegible] of [illegible] material requires a kind of physical setup which is not [illegible] available to us in building [illegible] on grounds of the Bethesda reservation.

Studies of Tumor (or Cancer) Viruses (See also "Some Approaches to Tumor Virus Research" attached)

Now a major research effort in the Virus Section, our studies of animal tumor and cancer viruses have been aimed at three unsolved problems that appear to us to be interfering most with our progress in tumor virus research.

The first problem is a technological one - the lack of simple virus techniques for studying most tumor viruses in the laboratory and for recognizing their occurrence in their natural hosts. Although signal advances have been made in the laboratory study on polyoma, Rous sarcoma, avian leukosis and Friend leukemia viruses, most tumor virus activity must still be assayed entirely by the production of tumors - a very slow, slow and uncertain method.

In 1959, Drs. Rowe, Hartley, Huebner, Kolin, White, and others in LID applied new viral techniques to studies of pathogenesis, modes of infection excretion, laboratory spread and natural occurrence to a number of viruses thought to be tumor agents - most notable polyoma virus, Friend leukemia, and the presumed avian lymphomatosis tissue culture agent.

Polyoma virus was shown to grow to extremely high titers in certain organs, 10 to 20 days after infection of the newborn mouse. Hemagglutinin, complement-fixing antigens, inhibitors and specific antibodies in addition to infectivity were compared in a number of different tissues and excretions at regular intervals from 3 days to 3 months after infections. Remarkable differences in titers of these various attributes were noted between various tissues. The most sensitive method for recovering virus from polyoma tumor tissues proved to be cultivation of the tumor tissue itself when infection was produced in infancy. Virus could often be demonstrated regularly beyond 3 months and in some mice appeared to persist throughout life.

Infection of weanlings led to significantly less multiplication of virus and excretion was minimal. Our serological group furnished information to other cancer research laboratories on the prevalence of spontaneous polyoma and extrinsic virus infections in their passage materials and breeding colonies.

and requires further developmental research.

of the country, thus assuring a high rate of association of antibodies

or at least gaining some control of this problem. However, it is a virus problem, requiring general competence in virology, and this is our specialty in LID.

The third critical need is information on the natural history of tumor viruses. Almost without exception contemporary tumor virus studies are confined to artificial laboratory study systems utilizing chiefly special categories of mice, chicken or tissue cultures. These various and quite valuable modes of approach to tumor virus study are meant to be "models" of what (it is hoped) happens in nature. This in part is frustrated by the "extrinsic" viruses. However, until the natural occurrences and biology of these agents are known, at least in part, their real importance as biologic entities and as causes of cancer must remain obscure.

By July, 1959 we had completed an extensive study of spontaneous infections with polyoma in numerous mouse breeding colonies used for study of mouse tumor viruses. Rowe and Hartley, working with Law, Andervont and many others, found that the amazing spread of polyoma infection in experimental colonies could be attributed to the close proximity of most such colonies to laboratory areas also used for newborn mice injected with tumor passage materials containing polyoma virus (in many cases the presence of the polyoma virus in the inoculum was unknown). Unexplained were evidences of extensive infections amongst breeding mice in some of the largest commercial colonies which were obviously not exposed to experimental work with polyoma of any kind Some other, perhaps "more natural" mode of spread had to be postulated.

Studies of Tumor Viruses in Nature

To answer these and other questions about the natural history of polyoma virus we decided in July, 1959 to do now what had to be done eventually, namely, study the occurrence and behavior of polyoma in Mus musculus, and in their natural environments, both rural and urban. Without exception, Mus musculus and other rodents in rural areas have so far failed to reveal any evidence of infection with polyoma. When the first 500 mice trapped in 43 different areas in lower Manhattan, Queens, Brooklyn and the Bronx showed widespread infections with certain other viruses but absolutely no evidence of polyoma, serious questions were raised about the origins and natural hosts of polyoma. This question was quickly resolved when our zoologist, Mr. Peterson, on the advice of Dr. David Davis (our consultant in medical zoology) turned to systematic surveys of mice in the more densely populated areas of Harlem. Here varying percentages of individual mice in more than half of the mouse populations surveyed were serologically positive. A field laboratory was immediately established in Harlem with the assistance of the New York City Department of Health. The story is quite incomplete - since the field laboratory had to be closed in October for lack of travel and field operating funds - however, the following was determined about the natural history of polyoma.

All newly developed techniques for assaying polyoma (HI, CF, VNP tissue culture isolation and neutralization techniques) confirmed the widespread presence of polyoma in the extremely dense mouse populations of certain tenement areas in central Harlem. In this area nearly 20% of approximately 1000 mice of all ages and sizes showed serological evidence of polyoma; polyoma virus was isolated from tissues and urine of 25% of the serologically positive mice, and at least two of the wild strains were shown to be highly tumorigenic.

Although arthropods (A. sanguineous, L. bacoti, and hair mites), and many insects (particularly roaches) were observed, often in great profusion, the studies failed to suggest that they played an important role either as reservoirs or vectors.

Polyoma virus was readily demonstrated in mouse nesting materials. This was consistent with our previous experimental observations that polyoma is excreted in large amounts from newborn mice, but inconsistent with our data on spontaneously infected adult laboratory mice which apparently did not. However, certain naturally infected adult wild mice excreted considerable amounts of polyoma. This information provided the basis for a hypothesis which could account for the natural spread of polyoma. This hypothesis recognizes the fact that certain mice, including adults, become chronic carriers and shedders of polyoma virus, an agent well equipped to survive outside the body. Also postulated were mouse populations large enough and dense enough to furnish a constant supply of susceptible mice, and an environment in which mice shared community nests contaminated with polyoma virus, all circumstances observed in the Harlem tenements. Since data previously reported from LID showed that mice could be infected easily by oral and respiratory exposures to polyoma virus, the hypothesis seems to satisfactorily explain much of the natural spread of polyoma. So that even in nature polyoma virus behaves much like an ordinary highly resistant respiratory or enterovirus. A number of rats and 60 humans living in the apartments containing heavy mouse and environmental infections with polyoma showed no definite evidence of specific polyoma antibodies. A number of mouse viruses were also encountered in the wild mice, including several related to common human viruses; and, as continues to be the case in nearly all laboratories studying tumor viruses (vide supra) these agents created many problems of a technical nature as well as difficulties in interpretating the data.

Comment

Early in the study it was clearly demonstrated once again that valid isolations of polyoma from field specimens could only be achieved outside our Bethesda laboratories which are demonstrably heavily contaminated not only with polyoma but other extrinsic murine viruses. The consequent necessity for doing much of the laboratory work in the field added unanticipated expenses to our field operations. Resumption and extension of these almost wholly unique studies of tumor viruses in natural hosts, in their natural environments, appears to us and many others almost mandatory. It is clear that studies of natural infection, similar to those described here are desperately needed if modern tumor virus research is to retain any concrete links with "real" experiences in nature. Yet it is also undesirable to attempt to begin studies that cannot be done properly or completed. Nowhere is the high cost of economy more apparent than in field studies designed to explore unknown and unpredictable phenomena but forced to depend on inadequate and inflexible support.

We have plans for resuming our natural history studies of polyoma, and for embarking on similar field studies of the natural distribution and modes of spread of other animal tumor viruses (and viruses presumed to cause tumors) for which reasonably simple and accurate assay methods are now available. Few, if any other research groups, have such studies in mind. We regard this as perhaps one of the better reasons for suggesting that exceptional efforts be made to support our pleas to be supported in this study.

[illegible] ...

The Mycology Section reported 7 projects for calendar year 1959. Although some aspects of all projects have been completed other aspects require further investigation and experimental study. All projects, therefore will be continued. Results of studies have been published or accepted for publication in 12 papers.

Dr. George W. Lones, with the assistance of Carl Peacock, induced resistance to Amphotericin B in Candida albicans although another investigator had reported failure in a similar study. Most of this work was completed in 1958 but cultures have been maintained and will be used in future studies of quantitative relationships of antagonism exerted on Aphotericin B by cysteine and sterols.

Dr. Lones, with the technical assistance of Mr. Peacock, has carried Coccidioides immitis in the spherule or parasitic form (through as many as 160 serial transfers in one series); has demonstrated a stimulating effect of CO_2 in maintaining this form of growth; has investigated the effects upon respiration of the spherule form C. immitis of pH, prior starvation and various substrates; and has used isotopically labelled glucose in determining amount of substrate assimilated. Quantitative studies have showed the roles of various carbon sources in supporting and favoring either saprophytic or parasitic growth forms.

Dr. Herbert Hasenclever, with the technical assistance of William Mitchell, has demonstrated partial protection against death (but not against infection) in mice immunized with formalin killed fungus cells and in mice infected with sub-lethal doses of Sporotrichum schenckii and subsequently challenged with normally lethal doses of this fungus. Similar studies with Candida and Cryptococcus are not yet completed. These studies are preliminary to future basic studies of immunologic processes in the mycoses.

Dr. Hasenclever has demonstrated experimentally in critically controlled quantitative studies that strains of C. albicans vary widely in virulence, that the mouse is as susceptible (w/w) as the rabbit to experimental candidiasis, and that some strains of C. stellatoidea are as virulent for mice as C. albicans. In other studies of virulence and pathogenesis of yeast-like fungi he has found that rate of multiplication in vivo of Cryptococcus neoformans is related directly to degree of virulence and that there is first an accumulation or retention of intravenously injected C. neoformans cells in the lungs, followed by an apparent decrease here in numbers of yeast cells, and an eventual rapid increase of their numbers in the brain.

In antigenic studies of yeasts, Dr. Hasenclever, with the technical assistance of Mr. Mitchell, has investigated by utilizing immune rabbit sera, the antigenic (and presumably the genetic)

[illegible] of [illegible] pathogenic [illegible]
Exophiala dermatitidis [illegible] with [illegible]
species of Candida. This [illegible] of [illegible]
of each species used and investigations using such strains will be
continued.

Dr. Chester W. Emmons, with the technical assistance of Willie Piggott and William Hill, has continued to find ecologic associations of pathogenic fungi with certain specific types of soil or organic material. Cryptococcus neoformans has been isolated from many additional sites where pigeons roost or nest. Virulent strains of the fungus have been isolated from attics of old open buildings and window ledges outside offline buildings. Detailed studies of microflora of Histoplasma positive soil samples taken adjacent to a building housing bats and comparative studies of Histoplasma negative soil samples taken a few feet away have not as yet revealed a recognized difference in fungal flora, except for presence or absence of Histoplasma.

Dr. Emmons and assistants have continued to test antifungal drugs in a standardized in vivo test using mice. Dr. Benjamin Prescott has collaborated by making and supplying drugs of one series which was systematically examined and has provided other drugs. Drs. John Utz and Vincent Andriole have collaborated by testing promising drugs in clinical trials. One antibiotic, found by Dr. Emmons 3 years ago to surpass other antibiotics in his experimental therapy studies, has now had encouraging clinical application.

In collaboration with the Bureau of Medicine and Surgery of the Navy, Dr. Emmons investigated the etiology of a febrile illness occurring in underwater swimmers at Key West. Evidence has been obtained which indicates that this may be an allergic manifestation of exposure to a fungus in persons already hypersensitive to this fungus. Several fungi were found growing in the masks used and three of these are still under investigation in experimentally hypersensitized animals and by field use of an antigen prepared from one of them. Experimental studies of 2 fungi and standardization of antigens prepared from them are incomplete. As a result of these unfinished studies better sanitation of the masks has been enforced and apparently has controlled the febrile disease.

Dr. Emmons, in collaboration with Dr. Lie-Kian-Joe, Dr. William Jellison and many other collaborators who send in pathological specimens for study or cultures of fungi for identification, has studied several very interesting and unusual pathogenic fungi. He and Dr. Lie, as a result of their studies and the unusual fungi isolated by Dr. Lie in Indonesia, have proposed the name "Phycomycosis" for diseases caused by fungi of the Phycomycete group, many cases of these infections being recognized only at autopsy after a specific identification of the etiologic fungus can not be made. Additional cases of the unusual mycosis caused by Basidiobolus ranarum have been found. In collaboration with Dr. Jellison taxonomic, cytologic and pathologic

(Section of Medical and Physiological Bacteriology)

The program of the Section on Medical and Physiological Bacteriology continues to be a varied and contemporary one. Emphasis is being placed a) on the mode of action of antibiotics and the development of resistance b) on the possibility of the development of a functional vaccine against group A streptococci and particularly an anti-M protein antibody, c) on mechanisms of the development of non-specific immunity and in particular the function of the iron transporting protein of plasma and its relationship to the potentiality of growth of pathogens in the body, d) on the possibility of modifying potent anti-fungal, anti-parasiticidal, anti-tuberculosis antibiotics in such a way that they will lose their toxicities, e) on the enzymology of the main energy-yielding reactions of bacterial cells and finally f) on the mechanism of hydrogen isotope fractionation brought about by a marine Pseudomonad. Several projects were terminated during the past year, including studies on the mode of action of aureomycin, transduction amongst the staphylococci, and studies on the amino acid composition of mycobacteria. Projects initiated during calendar year 1959 to replace these projects include study of staphylococcal penicillinase and a search for potential inhibitors of the enzyme, studies on the anti-microbial activity of marine water and a study of the electron transport system of autotrophic bacteria.

Dr. Roy Repaske, formerly Associate Professor of Bacteriology, Indiana University, Bloomington, Indiana has joined the unit on Microbial Physiology and is conducting studies on the electron transport system of the autotrophic bacteria. Dr. Fred D. Sisler, Geological Survey, joined this section as a Guest Worker. He is studying the biology and enzymology of fractionation of hydrogen isotope by marine microorganisms. During the year, the laboratories of Dr. Saz and Dr. Prescott were modernized, space was reallocated and a new laboratory was equipped for Dr. Repaske. The continuing source of regret concerning the program of this section is that authorization has not been forthcoming for the hiring of a microbial geneticist. This is the most important area of contemporary microbiology which is not represented in the program of the bacteriology section. Listed below are the projects currently being investigated by the various units of the section.

Summary of projects within the Medical and Physiological Bacteriology Section.

Studies on the biochemistry of antibiotics: A study is being made of staphylococcal penicillinase both in the whole cell and in cell-free extracts. Emphasis is being placed on the uncovering of an inhibitor for staphylococcal penicillinase. Since the penicillin nucleus is composed of cysteine and D-valine, it was thought possible that certain dipeptides could inhibit the activity of the enzyme. This has proved to be the case. Numerous dipeptides have been found to exert an inhibitory effect and, in particular, D-valyl D-valine has been found to inhibit strongly both cell-free and whole cell staphylococcal penicillinase. An offshoot of this work on the inhibition of

penicillinase [illegible] simple electrostatic attraction [illegible] rate of penicillinase activity. Indeed, stimulations in rate up to 50 fold have been obtained in the presence of n-propanol. As an operational hypothesis, it has been postulated that propanol is active by virtue of the removal of a naturally occurring inhibitor present in both whole cells and cell-free penicillinase. Attempts will be made to purify and isolate this putative inhibitor.

Since various terrestrial bacteria do not survive in sea water, a study has been initiated to determine the mechanism of the killing of these organisms, including pathogens. It has already been established that the high salt concentration of marine water is not responsible since both Escherichia coli and penicillin-resistant Staphylococcus aureus both grow rapidly in media made up with sea water in lieu of distilled water and even multiply when suspended in sea water without added nutrient. As a consequence, a search will be made for possible anti-bacterial substances present in sea water. Particular attention will be paid to water which shows high planktonic blooms, in particular, the so-called red tides. A collaborative project with the Woods Hole Oceanographic Institute is being discussed.

Studies on the iron-binding serum protein Siderophillin: The non-specific immune effect of blood serum as mediated by siderophillin on the growth and metabolism of selected bacterial pathogens is being intensively investigated. Particular attention is being focused on Staphylococcus aureus. The growth and metabolism of this pathogen is peculiarly affected by the percentage iron saturation of siderophillin in blood serum. The investigation extends to the factors leading to changes in concentration of both serum iron and siderophillin in circulating blood as a result of infection and other abnormal conditions and the consequence of such changes on resistance to infection. The function of siderophillin in the iron metabolism of the host and the mechanism by which mammalian tissues acquire from siderophillin the iron needed for normal growth and function are likewise being studied. The chemistry of the reactive groups in the siderophillin molecule responsible for the specific binding of iron and the physical chemistry of the association and dissociation of the metal protein complex are being investigated.

Detoxification of potential chemotherapeutic agents: Studies on the detoxification of tuberculostatic, parasiticidal and viricidal chemotherapeutic agents (Isoniazid and Streptomycin for tuberculosis, Mycostatin for fungal infections and Miracil D for parasitic infections) are being continued and extended. It has been observed that concomitant administration of certain calcium salts, steroids and combinations of sodium glucuronate and certain amino acids with the drugs are effective in increasing the tolerated doses in two strains of mice. A number of potentially useful compounds has been synthesized. Some of these have already demonstrated high in vivo activity in experimental animals against parasitic infections. Interestingly,

several of these compounds sent to the Cancer Chemotherapy Center have been initially screened and found to show significant anti-tumor activity with negligible toxicity for mice.

A collaborative project with Dr. William Steenken, Trudeau Laboratories, is under way. Dr. Steenken's group is studying the effect of various detoxified tuberculostatic agents on animal tuberculosis. In the event these compounds show promise, a collaborative project will be initiated with the Tuberculosis Division of the Veterans Administration for studies on the effect of suggestive compounds on human tuberculosis.

Studies on streptococci and other human bacterial pathogens: Investigations of the type specific immunity to Group A streptococci are being continued. A relatively new method for the isolation of M protein from streptococcal cells is being used. A streptococcal cell wall enzyme, lysin, is prepared from fresh phage lysates of Group C streptococci and is reacted with Group A streptococcal cell walls. The resulting digest, when fractionated by various means, including ion-exchange column chromatography yields an M protein which compares favorably in respect to antigenicity to the M protein extracted by conventional means. Type specific antibodies as demonstrated by various tests developed in rabbits after intracutaneous injection of 1.5 mg. per animal. Following such injections, no toxicity was noted. In the course of these studies, it was noted that lysin M as well as purified acid M and crude acid extracts from Group A streptococci contained a factor which precipitated mammalian plasmas and purified fibrinogens. This so-called FPF (fibrinogen precipitating factor) is under investigation to determine its biological significance. Further, the relationship between M protein and FPF activities is being studied. Thus far, they are inseparable. In addition, numerous vaguely defined areas presumptively related to M protein activity are being investigated. These include the clumping of streptococci in plasma and fibrinogen solutions, protection against phagocytosis by M protein, pathologic effects of M protein *in vivo*, fate of M protein *in vivo* and in leucocytes and transformation reactions among M types of Group A streptococci.

Studies previously conducted dealing with the upper respiratory tract bacterial flora of young children are now under statistical analysis. Laboratory work on this project was discontinued in June 1959.

Electron transport system in autotrophic bacteria: An intensive investigation is underway dealing with the electron transport system of various species of Hydrogenomonas. An attempt is being made to define and resolve the various components of the electron transport system in these organisms. Previous work on electron transport enzyme complex resolution has been hampered by the fact that the system is almost invariably bound to particles. A virtue of working with Hydrogenomonas is that at least a portion of the electron transport system is in a

soluble state. This project has no major findings to date since the investigator has just recently joined this section.

Microbiological fractionation of the hydrogen isotopes by a marine Pseudomonad: A guest worker in this Section, permanently assigned to the Geological Survey, has concerned himself with this problem. A marine Pseudomonad isolated from the Bahama Banks has been found to enrich with respect to deuterium as compared to light hydrogen to the extent of 20-fold. Similar results have been obtained using tritium. In both cases, the enrichment exceeds by far that which can be expected from exchange reaction equilibria. Warburg studies and biochemical analyses have shown that the initial fermentative degradation of glucose is by the Embden-Meyerhof scheme. Hydrogen arises presumably from glucose via formate. Formate is decomposed to CO_2 and H_2. One and perhaps two separate sites for deuterium discrimination has/have been fairly well delineated. One is the glucose-pyruvate step and the second occurs between formate and CO_2 and H_2. Practical implications of this study include the possibility of devising a cheaper, more efficient fractionation of the hydrogen isotopes.

Physical changes.

Several major construction jobs and general building changes were effected during calendar year '59 in an effort to meet the ever present laboratory-office space emergency and to provide more efficient service for building maintenance and operation. Many of these jobs have been carried to successful completion--such as conversion of telephone service to the NIH central switchboard, removal of air-conditioning equipment in Room 29; conversion of attic space to freezer storage space for storing biological specimens; installation of equipment to tie into central air-conditioning plant for expansion of air-conditioning facilities on three floors; redesign of corridor and lavatory space to create three offices for scientists; enlargement of sewer pipe lines to increase carry-off capacity; painting of Library, Lobby, and all office and corridor space and many more jobs of this nature. Unfortunately, the conversion of Room 29 to three laboratory-animal rooms has been a very costly venture, both in time and money. The contract required completion by September 28 and contained a penalty enforcement clause. Despite almost daily follow-up by our Administrative Office these three rooms are still unfinished. Some means should be found to require performance of the contract, which is now three months over-due.

Personnel.

Many personnel changes have taken place during the year. Eighteen new civil service employees (including two summer students), one Commissioned Officer, and two Research Associates entered on duty. Personnel wise we are pleased that we have been able to recognize the contributions of many employees by well deserved promotions; 23 grade changes, ranging from GS-2 through GS-14 were effected this year.

Awards.

Contributions of two employees were recognized through cash awards--one in the amount of $35.00 for development of a laboratory technique (with suggested submission in six months for additional award as benefits and savings accumulate) and the other for $200 for skill in the design of laboratory equipment.

Training.

Courses in outside training under sponsorship of NIH were completed by six employees and have already produced promising results.

Scientific exchange.

The exchange of scientific information at the international level is well represented by the Laboratory. Two of our scientists were sent abroad on collaborative studies in 1959 and will continue through the middle of 1960. Dr. Rosen, to Tahiti in conjunction with the University of California, to confer on problems of the epidemiology of filariasis and virus diseases, the other, Dr. Cook, to Paris, France to participate in research studies on

auto-immunity in viral infections and related virus research.

Two visiting scientists, Dr. Alexander Kohn from Israel, and Dr. David White from Melbourne, Australia--came to LID on fellowships. Dr. Kohn stayed approximately 8 months--left in December. Dr. White has been here since--plans to leave in June, 1960.

Problems in supporting field studies.

One of the major problems encountered administratively during the past year was the financing of various field studies from quarterly budget allocations. We have attempted to carry on this research without the necessary increase in funds. This method proved quite unsatisfactory during the past in that the field studies had to be cut back and finally given up altogether. In the coming year the Laboratory is hopeful that additional allocations can be made apart from the day-to-day operation costs to carry out specialized field programs. Supplementary requests will be prepared to justify our field studies. Apparently these are largely aimed at the solution of viral problems. Dr. Emmon's, however, has indicated that his developing program now calls for more field activities.

Serial No. NIAID 60
1. Infectious Disease
2. Medical & Physiol
Bacteri
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Reversal of Antibiotic Activity of Aureomycin by Culture Filtrates of Pseudomonas Species.

Principal Investigator: Dr. Arthur K. Saz

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 7/12
Professional: 2/12
Other: 5/12

Project Description:

Objectives: To investigate the anti-Aureomycin activity of culture filtrates of various Pseudomonas species. To purify, isolate and identify compound(s). To determine relationship, if any, to mode of action of Aureomycin.

Methods Employed: Standard bacteriological and such biochemical techniques as may be indicated.

Major Findings: The reversing factor has been partially purified and its specific activity as measured by its ability to reverse the antibiotic activity of a given concentration of Aureomycin has increased. The factor is a relatively small molecule.

Proposed Course of Project: The degree of reversal of Aureomycin activity afforded by this compound is small. Further the amount present in a Pseudomonas culture is extremely small and does not seem, as a consequence, to warrant further more detailed investigations. This project consequently has been terminated.

Part B not included

Serial No. NIAID 60-4
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Mode of Action of Aureomycin and Biochemical Mechanism of Resistance Thereto.

Principal Investigator: Dr. Arthur K. Saz

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 12/12
Professional: 3/12
Other: 9/12

Project Description:

Objectives: To determine the biochemical basis for the inhibition of sensitive bacteria by Aureomycin, and the biochemical mechanism of resistance of bacteria to the antibiotic. To study methods of increasing the sensitivity of Aureomycin-resistant bacteria to the antibiotic.

Methods Employed: Isolation, resolution and purification of various electron transport enzymes from both sensitive and resistant E. coli will be carried out.

Major Findings: It has been determined that the electron transport system stimulating factor present in serum albumin and other, but by no means all, proteins is indeed the protein. The purified isolated protein, in addition to stimulating electron transport reactions in E. coli, also increases sensitivity of electron transport enzyme complex to Aureomycin. The various proteins have

Part B included: Yes

been chromatographed and electrophoresed and in each instance the active factor strictly follows the protein content of the various fractions.

Proposed course of project. This project has been terminated. As a result of work on this project over the past six years, one may speculate profitably on the mode of action of Aureomycin and the biochemical mechanism of the development of resistance thereto.

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PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Saz, Arthur K. and Martinez, L. M. Enzymatic basis of resistance to aureomycin. III. Inhibition by aureomycin of protein-stimulated electron transport in Escherichia coli. Accepted for publication in the Journal of Bacteriology.

Honors and awards relating to this project:

Dr. Arthur K. Saz:

Participated in the Course on Military Medicine and Allied Sciences at the Walter Reed Army Institute of Research -- presented lecture-seminar "Mechanisms of antibiotic action."

Invited to conduct seminar "Studies on the mechanism of action of aureomycin and the development of resistance thereto" at Duke University School of Medicine.

Invited to serve as convener of the session on Electron Transport at the annual meeting of the Society of American Bacteriologists.

Invited to be charter member, American Academy of Microbiology.

Appointed Chairman, President's Fellowship Committee, Society of American Bacteriologists.

Serial No. NIAID 60-B
1. Infectious Diseases
2. Medical and Physiol... Bacteria...
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Autolysis in Genus Bacillus. Mechanism of Cysteamine-induced Lysis in the Genus Bacillus.

Principal Investigator: Dr. Arthur K. Saz

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)

Total:	3/12
Professional:	1/12
Other:	2/12

Project Description:

Objectives: A study of the biochemical mechanism of cysteamine-induced lysis of Bacillus subtilis.

Methods Employed: Preparation of cell-free extracts by various means, purification by paper and column chromatography and electrophoresis and other methods as may be indicated by results obtained. Standard bacteriological techniques.

Major Findings: No new major findings.

Proposed course of project: This project has been terminated. Due to the extreme variability of Bacillus subtilis strains in culture (as reported in last year's annual report), it has been impossible to secure consistently reproducible results. Attempts to stabilize morphological and biochemical properties of these organisms by various means and by genetic studies have proven fruitless.

Part B. not included.

Serial No. NIAID 61
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Cellular and Cell-Free Staphylococcal Penicillinase

Principal Investigator: Dr. Arthur K. Saz

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 18/12
Professional: 5/12
Other: 13/12

Project Description:

Objectives: To purify and study penicillinase from penicillin-resistant staphylococci. To investigate the possibility of strongly inhibiting cellular penicillinase.

Methods Employed: Isolation and purification of cell-free penicillinase from staphylococcal cells by standard biochemical techniques. Measurement of penicillinase activity manometrically and iodometrically.

Major Findings: The organism used contains a non-inducible, non-diffusible penicillinase and is resistant to greater than 400 µg penicillin per ml. A major task to date has been to find methods of extracting the enzyme from the cell without denaturing the protein. It has now been established that a Nossal disintegrator yields an acceptable product. Efforts are being made to secure a highly purified preparation. To date, four-fold increase in specific activity has been obtained.

Since the activity of penicillinase on penicillin is essentially that of a peptidase (hydrolysis of the β-lactam ring to yield penicilloic acid) it was thought possible that various amino acids and dipeptides would exert an inhibitory effect on the hydrolysis. A large number of highly purified L & D-amino acids and dipeptides resolved completely into the four possible enantiomorphs (L,L; L,D; D,L and D,D) has been obtained through the courtesy of Drs. M. Winitz and S. Birnbaum, NCI. Several peptides and derivatives have been found to inhibit

cell-free penicillinase markedly. The most active is D-valyl D-valine which inhibits up to 70%. The other three enantiomorphs are inactive. This finding is of particular interest since the penicillin nucleus is composed of L-cysteine and D-valine and thus inhibition by D-valyl D-valine may represent competition for active site on the enzyme surface between substrate (penicillin) and inhibitor. Other D-valine derivatives and analogs also show effects. Certain L-threonine containing peptides are also inhibitory.

Numerous metal-binding agents tried have no effect on penicillinase activity, but as a by-product of this aspect of the problem, a remarkable effect of alcohols on activity has been observed. Stimulations of up to a 3500% in rate of enzyme activity have been obtained by adding n-propanol to the reaction mixture. Other alcohols have lesser effects and the effect seems to fall in a series relating to the molecular weights of the alcohol, i.e. methyl < ethyl < n-propyl > isopropyl > butyl > amyl. Similar stimulations are recorded using whole cells as penicillinase preparations. A particularly interesting observation from the theoretical point of view is that an inactive fraction obtained from purification procedures shows activity in the presence of n-propanol. It is possible that a latent penicillinase is present in laboratory-induced penicillin-resistant staphylococci (which as distinct from clinically isolated resistant staphylococci never show penicillinase activity). This is under investigation.

It is conceivable that n-propanol and other alcohols are stimulatory because they remove a potent naturally occurring penicillinase inhibitor. This too is under further investigation.

Significance to bio-medical research and the program of the Institute: If a non-metabolizable non-toxic inhibitor of staphylococcal penicillinase could be found it would go a long way toward solving the penicillin-resistant staphylococcus problem. Any information concerning penicillinase is of interest to this institute.

Proposed course of project: 1. To purify penicillinase further and to study its properties. 2. Co continue the search for an effective inhibitor. Animal studies will be used as indicated. 3. To study the mechanism of the alcohol stimulation of penicillinase.

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1. Infectious Dis[illegible]
2. Medical & Physiolog[illegible]
Bacteriol
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Antibiotic Activity of Sea Water

Principal Investigator: Dr. Arthur K. Saz

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 2/12
Professional: 1/12
Other: 1/12

Project Description:

Objectives: To investigate the reported antibiotic activity of sea water. To isolate from sea water, compounds active against fecal organisms and penicillin-resistant staphylococci.

Methods Employed: Treatment of sea water by various methods (extraction, chromatography, electrophoresis) in order to isolate inhibitory factors (if any) present. Standard bacteriological techniques

Major Findings: As yet, it has not proved possible to confirm the widely accepted thesis that sea water is toxic to usual terrestrial organisms. Culture media composed of 90% sea water and 10% distilled water supports the growth of Escherichia coli and S. aureus (penicillin-resistant) as well as media prepared in 100% distilled water. Further organisms of the two genera suspended in sea water with no added nutrients will grow rapidly after a slight initial decrease in numbers.

Significance to bio-medical research and the program of the Institute: The public health aspects of the mechanism of the killing of fecal organisms in sea water are obvious. An inhibitor of penicillin-resistant staphylococci would be of prime importance.

Proposed course of project: A cooperative arrangement with Woods Hole Oceanographic Institute will be arranged for collection of sea water for examination. Particular attention will be paid to samples collected during planktonic blooms, such as so-called red tides. A search will be made for substances in sea water supporting the growth of bacterial species, as indicated above.

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1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The electron transport system in an autotrophic hydrogenomonad

Principal Investigator: Dr. Roy Repaske

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 7/12
Professional: 4/12
Other: 3/12

Project Description:

Objectives: This research has as its object the identification of individual enzymes and intermediary cofactors which are operative in electron transport. This information may be transposable in part to mitochondrial systems and provide important clues about the electron transport sequence in these particulate structures.

Methods Employed: The methods employed will be those necessary for mass cultivation of organisms and for isolation and purification of the electron transport enzymes. Growth of large quantities of Hydrogenomonas eutropha is technically difficult because of the requirement for large volumes of an artificial gas mixture. Apparatus has been designed and is being built for this purpose. The assay of the initial enzymatic reactions in cell free preparations also requires a modified procedure because the enzymic activity must be measured in a hydrogen atmosphere in a spectrophotometer. Protein fractionation will be achieved by procedures involving ammonium sulfate precipitation, gel adsorption and elution, solvent precipitation, and chromatography.

Part B not included.

Major Findings: There are no major findings to report because the principal investigator has recently transferred from another institution.

Significance to bio-medical research and the program of the Institute: Hydrogenomonas species are unique organisms which obtain energy for all of their life processes through oxidation of hydrogen gas in the presence of oxygen. This is in contrast to animal cells and most bacteria which oxidize organic substances for energy. Although the initial substrates are different, there is every reason to believe that both types of systems have much in common. Both serve the identical function of providing energy for synthesis, growth and repair, and both utilize cytochromes and diphosphopyridine nucleotide to mediate the transfer of electrons to oxygen. Biochemical studies in the past have revealed that a common denominator exists between biochemical processes of various organisms, and this well supported concept of comparative biochemistry is the rational basis for investigating a given reaction in an organism most amenable to study.

Previous research on this organism was carried out at another institution by the principal investigator. The potential advantage of studying these reactions in H. eutropha lies in the fact that the system in this organism is soluble and therefore it can be fractionated by classical protein fractionation procedures.

The electron transport system in all other organisms studied to date has been associated with particulate elements of the cell. Efforts to dissociate some of these enzymes from the particles have met with limited success while other enzymes cannot be solubilized. Concomitantly with the occurrence of electron transport, ATP is generated; of the two processes, ATP formation is the most labile during manipulation. As a result of these characteristics, our knowledge of electron transport and simultaneous ATP formation is sketchy and indirect.

Proposed course of project: During the next year attempts will be made to isolate the principal enzymes in the electron transport sequence and to obtain them in reasonably purified form. Cofactors and intermediate acceptors in these reactions will also be studied.

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1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Microbiological Fractionation of the Hydrogen Isotopes

Principal Investigator: Dr. F. D. Sisler

Other Investigators: Dr. Micah Krichevsky

Cooperating Units: U. S. Geological Survey

Man Years (calendar year 1959)
Total: 17/12
Professional: 9/12
Other: 8/12

Project Description:

Objectives: To determine the mechanism of hydrogen isotope fractionation by microorganisms; the divergent metabolic pathways of protium, deuterium and tritium; the effect of the heavy hydrogen isotopes on the physiology of the simple biological systems; the ecological and evolutionary significance of the isotope effect in the biosphere.

Methods Employed: The metabolism of whole cells, sonically disrupted cells and enzyme extracts of a marine pseudomonas were studied in the presence of isotope mixtures. Liquid metabolic products of systems were analyzed by mass spectrometer, infra red and radioactive tracer methods and conventional biochemical analytical procedures.

Part B included: Yes

Major Findings: The isotope fractionation factor with respect to the equilibrium:

$$H_2O + HD \rightleftharpoons H_2 + HDO$$

ranges from D_2/H_2 = 1/26,000 - 1/33,000 to 1/134,000 with the organism tested.* No inhibition exists where the concentration of D_2O is less than 25% in the various media examined. The fractionation factor, where protium in the gas phase of the hydrogenylase-containing pseudomonas is in excess by a factor of five above the initial equilibria appears to be consistent with that of the theoretical bond cleavage rates at 25°C:

$$\frac{O\text{-}H}{O\text{-}D} = 10.6$$

However, the enrichment factor of 20 (D_2/H_2 = 1/34,000) suggests at least a two-step process of deuterium discrimination (normal sea water = 1/6,700).

Where tritium has been substituted for deuterium, the enrichment factor, at least for short periods of time, increased beyond a one-step theoretical maximum. Thus, media containing from 0.005 to 5.0 μc/ml of HOT have more than 50% of tritiated water molecules removed from the medium within several days. This exceeds by far what can be expected from the exchange reaction equilibrium.

Studies with tritiated water indicate that this pseudomonad has a water pumping rate of 10^{13} molecules H_2O/hour/cell at 25° C when exposed to an excess of energy source during the phase of maximum metabolic activity as measured by hydrogen production.

The stages of tritium transfer appear to be HOT => cell => polysacharride => HOT. Tritium eventually reequilibrates with the water of the medium after passing through the cell. The output tritium fraction appears to be either glycogen or a related polysacharride.**

* Analyses by Dr. Irving Friedman, U.S. Geological Survey

** Analyses by Dr. Benjamin Prescott, LID-NIAID

Warburg studies and biochemical analyses show that the initial fermentative degradation of glucose is by the Embden-Meyerhof scheme.* Glucose is degraded to formate and finally to CO_2 and H_2. One, and perhaps two, separate sites for deuterium discrimination has/have been fairly well delineated. One is the glucose to pyruvate step and the second between formate and CO_2 and H_2.

Significance to bio-medical research and the program of the Institute: A knowledge of hydrogen metabolism of biological systems can be considered as one of the most fundamental of metabolic processes. Hydrogen metabolism (hydrogen transfer, electron transfer) is synonymous with the energetics of living processes.

With increasing use of both natural and radioactive isotopes in medical research, it is considered of utmost importance that attention be focused on fundamental studies which may contribute to the understanding of the isotope effects in biological systems. This study of the hydrogen isotopes in simple cell metabolism should, therefore, contribute basic information of value towards the solution of problems of public health and economic importance such as implications in radioactive waste disposal in marine environments, toxic effects of deuterium and other isotopes and further is of fundamental importance in studying new metabolic pathways in microbial and other systems.

Proposed course of project: To continue current research with the following objectives in view:

1. Isolation and identification of intracellular compounds primarily responsible for deuterium and tritium fractionation.

2. Examination of extracellular products showing heavy isotope enrichment.

3. Evaluation of the chemical thermodynamics involved in isotope fractionation, comparing experimentally established values with the theoretical.

4. Further studies on transport of isotopes across cell membranes; rate processes and steady state equilibria of all phases involved in isotope fractionation.

* Warburg studies by Dr. Micah Krichevsky (LPC-NIAID) and Miss Marcia Newell, U.S. Geological Survey.

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Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Sisler, F. D. Biogeochemical concentrations of deuterium in the marine environment. Science, 129:1288, 1959.

Honors and Awards relating to this project:

Dr. Frederick D. Sisler:

Presented invitation paper "Biogeochemical concentration of deuterium in the marine environment" at the 96th annual meeting of the National Academy of Sciences.

[illegible] (6)
1. Infectious Diseases
2. Medical & Physiological
Bacteriology
3. Bethesda, Md.

[illegible]
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Non-specific Immunity Associated with Serum Siderophilin and the Iron Metabolism of both Pathogen and Host

Principal Investigator: Dr. Arthur L. Schade

Other Investigator: Dr. Lathrop Roberts

Cooperating Units: None

Man Years (calendar year 1959)
Total: 25/12
Professional: 14/12
Other: 11/12

Project Description:

Objectives: To investigate the contribution of blood serum siderophilin to the natural resistance of the host; to investigate the iron requirements of selected pathogens and the effect of the iron-chelating siderophilin and conalbumin on in vivo iron accumulation, growth, and metabolism of these pathogens; to investigate the factors leading to changes in concentration of both serum iron and siderophilin in circulating blood as a result of infection and other abnormal conditions, and the consequence of such changes on resistance to infection, iron absorption by the host, and anemia.

Methods Employed: Bacterial and cultural procedures in specially deironized media; spectrophotometric and radioisotopic methods; metabolic and enzymatic analytical procedures; and methods particularly suited to estimation of iron chelated either to siderophilin or conalbumin and to their relative unsaturations.

Part B included: Yes

Major Findings: On the basis of previous findings showing that many bacteria will not grow in serum unless the contained siderophilin is saturated with iron, the conclusion was drawn that with respect to such bacteria the iron nutritional condition obtaining in the blood (extremely low concentration of free iron) provided an effective non-specific immunity barrier to infection by those bacteria irrespective of other possible specific or non-specific immunity factors. In addition, the rate of growth of some bacteria is decreased by the presence of unsaturated siderophilin. Similar results are obtained when the iron-binding conalbumin of egg white is present in the medium.

Quantitative study of the effect of the percentage of iron saturation of siderophilin on the rate of growth of Staphylococcus aureus in human serum under aerobic conditions has revealed the following significant facts:

1. the length of the observable lag period of growth is a function of the percentage iron saturation, e.g. at 136% saturation (iron excess) the lag period was 7½ hours; at 90% saturation the lag period was extended to 18 hours; at 50% saturation the lag period was 26 hours; and at 30% saturation it was 27 hours.

2. the rates of growth given as the slopes of the curves illustrating increase of turbidity ("d" values) per hour were: 0.490 for 136% saturation; 0.405 for 90%; 0.102 for 50%; and 0.050 for 30% iron saturation; thus, the percentage iron saturation of siderophilin as found in sera of normal subjects and patients suffering various disorders affecting their serum iron level and total iron-binding capacity will be one determining factor in the host's ability to resist infection by Staphylococcus aureus.

To establish the generality of the differential growth-inhibiting effect on different strains of Staphylococcus albus and S. aureus by human serum in terms of its iron-saturated siderophilin levels, we investigated the growth of 16 strains of S. aureus (6 penicillin resistant - coagulase positive; 5 penicillin resistant - coagulase negative; 5 penicillin sensitive - coagulase positive) and 4 strains of S. albus (3 penicillin sensitive - coagulase negative; 1 penicillin resistant - coagulase negative) in samples of human serum 42% and 109% iron saturated. All strains of S. aureus grew in the 42 and 109% iron saturated sera, with enhanced growth in the 109% iron-saturated medium. No S. albus strains grew in the 42% iron-saturated serum but did grow when the siderophilin was iron-saturated. Hence, the growth inhibitory effect of siderophilin

on the Staphylococci shows no correlation with the penicillin sensitivity or the coagulase property of the strains studied but rather expresses, probably, a more general metabolic and nutritional characteristic common to strains of both species. Worthy of note is the indicated value of siderophilin (or conalbumin) as a specific diagnostic medium factor to distinguish Staph. aureus from Staph. albus.

Preliminary studies have been made on metabolic and biochemical characteristics of Staphylococcus aureus grown in media, either serum or trypticase plus egg white, at different iron saturations of the appropriate iron-chelating protein. From such cultures we have obtained S. aureus cells with high and low iron contents. Thus far we have examined such cells for possible differences in cytochrome composition; catalase activities; oxidative capacities on glucose, succinate, and acetate; aerobic and anaerobic glycolytic as well as dehydrogenase activities. Comparisons have been made between cells grown aerobically and anaerobically as to these characteristics. In general, the results to date suggest that S. aureus cells grown aerobically under conditions of iron restriction resemble cells grown anaerobically. More work is planned to make the comparisons definitive. The hemolytic activity of the high and low iron cells and their products has also been preliminarily investigated.

We have extended our investigation into the usefulness of employing S. aureus as a tool for assessing the integrity and nativity of pure isolated human siderophilins. Samples of siderophilin produced under Red Cross auspices, some produced by the Protein Foundation and employed clinically by Dr. Crosby of Walter Reed Hospital, and samples of this serum protein isolated by Prof. Schultze of the Behring-Werke have been subjected to our tests. Only 2 recently acquired samples from the Behring-Werke have controlled the iron uptake by S. aureus cells in a manner comparable to fresh serum. Further study is needed to assure ourselves of the validity of this test for isolated siderophilins.

Significance to bio-medical research and the program of the Institute: The study of the naturally occurring iron-chelating serum protein, siderophilin, contributes to our understanding of the non-specific immunity mechanism available to humans for protection against microbial infection. Investigation of the qualitative and quantitative effects of siderophilin on the growth and metabolism of the pathogen Staphylococcus aureus will provide useful information on the pathogenicity of this bacterium especially under conditions closely approximating in vivo conditions.

Proposed Course of Project: We shall investigate in detail the growth, metabolism, and enzymatic capabilities of S. aureus when grown under conditions approximating the in vivo state with especial regard to the effect of different serum iron-saturation levels on

such characteristics. Additional biological features such as hemolysis, effectiveness of phagocytosis, and possible differences in response to antibiotics of high and low iron-containing cells will be studied. Preliminary investigations on the bacteriophagy of S. aureus as affected by the iron-saturation of the serum medium are also contemplated.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Schade, Arthur L. Mineral metabolism, with special reference to iron utilization. The Journal of the New York Medical College, Flower and Fifth Avenue Hospitals, 1:80-84, 1959.

Schade, Arthur L. and Stengle, James M. Effect of phenylhydrazine on the siderophilin and iron levels in sera of rabbits. The Jour. Lab. and Clin. Med., 54:593-598, 1959.

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1. Infectious Diseases
2. Medical & Physiolo
Bacteriol
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Chemical Aspects of the Specific Binding of Iron to Serum Siderophilin and Egg White Conalbumin.

Principal Investigator: Dr. Robert C. Woodworth

Other Investigators: Dr. Arthur L. Schade

Cooperating Units None

Man Years (calendar year 1959)
Total: 23/12
Professional: 11/12
Other: 12/12

Project Description:

Objectives: The goals of this project are to investigate the chemistry of the reactive groups in the siderophilin molecule responsible for the specific binding of iron and to determine the physico-chemical means by which the iron-siderophilin complex can be dissociated under physiologic conditio s obtaining in pathologic and normal hosts.

Methods Employed: Methods include the employment of immunological assays for purity and for content of the iron-binding proteins, radioactive tracers, spectral analyses, ion-exchange chromatography, electrophoresis, polarography, and polarimetry.

Major Findings: The facts, previously reported, that all the bound iron of siderophilin or conalbumin is precipitated by the appropriate immune serum (as determined by tracing the iron with and that siderophilin and conalbumin are antigenically distinct, hav

Part B included: Yes

were utilized in a detailed study of the kinetics of dissociation of the iron-siderophilin complex. Fresh human sera were used as sources of siderophilin because our studies have shown most purified siderophilin samples to be significantly different from siderophilin in serum in regard to their iron-binding and releasing properties.

Although under physiologic conditions of pH and ionic strength iron is so tightly bound to siderophilin that no free ionic iron is demonstrable, the iron-siderophilin complex does indeed dissociate at a finite, measurable rate. One approach to these rate measurements has involved the transfer of iron from siderophilin to conalbumin.

Reaction: siderophilin-Fe_2^{59} + conalbumin --> siderophilin + conalbumin-Fe_2^{59}

Assay: Ab + conalbumin-Fe_2^{59} --> Ab-conalbumin-Fe_2^{59} ↓

(Ab: antibody)

In varying successively the concentrations of iron-siderophilin and conalbumin, we established that the reaction showed first order dependence on the siderophilin concentration and zero-order dependence on the conalbumin concentration and had a specific rate constant of 2.8×10^{-5} $sec.^{-1}$

The other approach to the dissociation rate of the iron-siderophilin complex has been through the study of iron isotope exchange.

Reaction: siderophilin-Fe_2 + 2 Fe^{59} --> siderophilin-Fe_2^{59} + 2 Fe

Assay: Ab + siderophilin-Fe_2^{59} --> Ab + siderophilin-Fe_2^{59} ↓

After requisite mathematical treatment the data were plotted and showed an unexpected result. Rather than the expected continuous straight line, two intersecting straight lines were observed with the break occurring at half-reaction. By varying successively the concentrations of iron-siderophilin and free excess iron, we established that the steep initial line showed first order dependence on the iron-siderophilin concentration, zero-order dependence on the free iron concentration, and had a specific rate constant of 2.8×10^{-6} $sec.^{-1}$. The second, less steep, line showed no change in slope with various dilutions of the system and with a constant ratio of iron-siderophilin to free iron. An interpretation consistent with the above facts is represented in the following equations:

$$Fe\text{-}{}_a\text{siderophilin}_b\text{-}Fe \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} Fe^{3+} + {}_a\text{siderophilin}_b\text{-}Fe$$

$${}_a\text{siderophilin}_b\text{-}Fe \xrightarrow{k_2} {}_a\text{siderophilin}_b + Fe^{3+}$$

$$k_2 > k_{-1} \gg k_1$$

Preliminary studies of the pH dependence of the iron-siderophilin dissociation have shown the rate to increase markedly at pH s below 7.5. The studies employed isotope exchange as described above.

Preliminary studies of the rate of corbination of iron with siderophilin indicate the reaction to be extremely rapid. Careful quantitative measurements of these rates may require special techniques. Spectrophotometry has been used thus far.

Significance to bio-medical research and the program of the Institute: The shifts in bound iron levels occurring as a result of infectious diseases, acute infections, and allergic reactions are recognized. The availability of plasma iron to bacterial pathogens is a function of the siderophilin molecule's affinity for iron and of its degree of iron-saturation. Knowledge of the chemical bases of this affinity should elucidate the manner in which siderophilin governs the transfer of iron to the host as well as to the pathogen.

Proposed course of project: Completion of the studies of the association kinetics of iron with siderophilin will yield data which, together with the already determined dissociation rates, will enable us to describe the equilibrium between the iron-siderophilin complex and siderophilin plus free iron. Polarographic studies of the iron siderophilin complex will provide a check on the results derived from the kinetic studies. An investigation of the tightness of binding of bicarbonate ion to the iron-siderophilin complex will indicate what the role of bicarbonate may be in iron release and binding by siderophilin.

Studies will be made of the possibility of applying these in vitro methods to the evaluation of the integrity and nativity of isolated siderophilin serum fractions intended for use in chemical studies.

Serial No. NIAID 63-A

PHS-NIH
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Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Woodworth, Robert C. and Schade, Arthur L. Conalbumin: A Rapid, High-Yield Preparation from Egg White. Archives of Biochemistry and Biophysics, 82:78-82, 1959.

Serial No. NIAID 5?
1. Infectious [illegible]
2. Medical & Physi[illegible]
Bacteri[illegible]
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemistry of the acquisition of iron by mammalian tissues as mediated by the iron-binding protein, siderophilin.

Principal Investigator: Dr. Arthur L. Schade

Other Investigators: Dr. Robert C. Woodworth

Cooperating Units: None

Man Years (calendar year 1959)
Total: 17/12
Professional: 5/12
Other: 12/12

Project Description:

Objectives: To investigate the mechanism by which mammalian tissues acquire from serum siderophilin the iron required for normal growth and function and to study the effect of physiological conditions upon this exchange.

Methods Employed: Hematological procedures, radioactive tracer techniques, manometric techniques and immunologic analyses.

Major Findings: Reticulocytes remove iron from siderophilin up to 67 times as fast as the natural dissociation rate of iron-siderophilin complex under physiologic conditions of pH and ionic strength. The rate of removal of iron from siderophilin by reticulocytes is independent of percentage saturation of siderophilin. Both iron atoms are removed by reticulocytes from this chelating protein even in the presence of excess ionic iron while iron chelated with conalbumin or complexed with ethylenediamine tetraacetic acid (versene) or in an ionic form is not removed or taken up at significant rates.

Cell-wall-free reticulocyte lysate catalyzes the transfer of iron from siderophilin to conalbumin at the same rate that a comparable

[illegible] of the pH from physiological values [illegible] rate of uptake of iron from siderophilin by rabbit cells at the same pH shift markedly increases the rate of removal [illegible] from the iron-protein complex.

Significance to bio-medical research and the program of the Institute: As a first approximation, the normal functioning of host tissues requires iron for synthesis of their iron enzymes. Hence, the conditions of availability of serum iron to host tissues and the mechanism of its transfer from siderophilin are of significance to host-parasite relationships obtaining in infections.

Proposed course of project: We propose to extend our preliminary findings to establish the basis of the indicated catalysis of iron exchange by bone marrow and reticulocytes with iron siderophilin and to determine whether such a mechanism applies to non-erythropoietic tissues as well. Tissue culture, if feasible, may also be studied for this purpose.

Serial No. NIAID 64
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Detoxification of Potential Tuberculostatic, Fungistatic, Parasiticidal and Viricidal Agents.

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: None

Cooperating Units: Section on Chemotherapy, LPC-NIAID
Dr. George Luttermoser NIAID No. 131-C

Man Years (calendar year 1959)
Total: 15/12
Professional: 5/12
Other: 10/12

Project Description:

Objectives: Further studies on toxicity and detoxification of the tuberculostatic drugs, streptomycin (SM), isoniazid (INH) and mixtures of streptomycin-isoniazid (SM-INH) in two strains of mice. To find further means of increasing the tolerated doses of these widely used compounds and to find means of reducing the toxicity of several parasiticidal drugs.

Methods Employed: The effect of various steroids, sodium glucuronate, glycine and calcium salts on the toxicity of INH and/or SM was measured by the effect on survival time of normal mice following simultaneous administration of a given chemical with a lethal dose of drug. The effect of adjuvant on blood plasma levels of INH was measured by the "pyridyl" method.

Part B included: Yes

Major Findings: 1. Sodium glucuronate and glycine with SM-INH mixtures: 25 mg. of each of these two detoxifying agents as adjuvants permitted 89 of 90 mice (99 per cent) to tolerate a lethal mixture of 6 mg INH and 5 mg SM when administered subcutaneously. With 50 mg of each adjuvant 37 of 40 mice (93%) tolerated 8 mg INH and 10 mg SM, 100% lethal per se.

2. Steroids with INH, SM and INH-SM mixtures: Further studies have included the use of 9 steroids at various dosage levels as possible detoxifying agents for SM, INH and with various mixtures of SM-INH in two strains of mice. Four of the steroids permitted two strains of mice to survive double the lethal dose of SM. When injected simultaneously with 50 mg cholic acid, the SM dose may be increased to 40 mg, 4 times the lethal dose. With 10 mg of sodium taurocholate, sodium glycocholate or sodium glycotaurocholate, the results were very similar, 20 mg of SM could be tolerated by 100% of both white and DBA mice. In chronic toxicity tests, mice tolerated 20 mg of SM. However, necrotic areas were observed at the site of injection. With INH alone, cholic acid and sodium taurocholate showed slight detoxifying action.

3. Steroids with SM-INH mixtures: The four steroids have been tested as adjuvants with various mixtures of the two therapeutic agents in two strains of mice. With the four steroids at levels of 25 mg per 20 gram mouse, 100% of the mice tolerated administration of the toxic mixture, 20 mg SM and 2 mg INH.

4. Calcium lactate with SM-INH mixtures: Since Ca lactate proved effective in permitting mouse survival when administered simultaneously with a toxic dose of SM, the detoxifying action of this compound was tested on SM-INH mixtures in two strains of mice. 100% of the mice tolerated a lethal mixture of 10 mg SM and 6 mg INH when injected simultaneously with 12.5 mg of the adjuvant. Decreasing the dose of adjuvant to 6.25 mg permitted 85% survival of white mice and 80% of DBA animals with this mixture. With a 25 mg/20 g mouse dose of Ca lactate, 89% of the mice tolerated a 20 mg SM and 2 mg INH mixture. In chronic toxicity tests, 80 to 90% of the mice tested tolerated repeated injections of the SM-INH mixture for 15 daily doses.

5. With Miracil D: Various sulfonic acids have been tested orally simultaneously with this drug. Three acids, metanilic acid, orthanilic acid and dl-10 camphorsulfonic acids showed excellent detoxifying action. 30 mg of Miracil D/20 gram mouse (6 x lethal dose) was tolerated by 100% of the mice when administered by gavage simultaneously with either 50 mg of metanilic, orthanilic or dl-10 camphorsulfonic acids. As with 6 thymolsulfonic, previously reported, mice tolerated repeated daily administration of the mixture.

6. With Hetrazan: The use of various solvents as possible detoxifying agents for this drug increased mouse tolerance to the compound. Polyethylene glycol #100, #200 or #300 (50%) permitted 90-100% survival of DBA mice following a single oral administration of a lethal dose of Hetrazan. Increased amounts of the chemotherapeutic agent were better tolerated in 50% polyethylene glycol solution than in water.

Significance to bio-medical research and the program of the Institute: Increase of tolerance to any chemotherapeutic agent, whether by reducing toxic manifestations or by permitting larger dosage without loss in therapeutic activity should result in more effective therapy.

Proposed course of project: Animals will be experimentally infected with appropriate organisms and the therapeutic efficiency of detoxified compounds will be studied. In addition, further studies on a colorimetric determination for the tuberculostatic antibiotic, kanamycin, will be carried out.

Serial No. NIAID 61

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Prescott, Benjamin, Kauffmann, Gladys, James, Walter D. and Stone, Herald J. Means of increasing the tolerated dose of streptomycin in mice. II. Certain vitamins. Antibiotics and Chemotherapy, 9:369-375, 1959.

Prescott, Benjamin, Kauffmann, Gladys and Stone, Herald J. Means of increasing the tolerated dose of isoniazid-streptomycin mixtures in mice. II. Certain vitamins. Accepted for publication in Antibiotics and Chemotherapy.

Serial No. NIAID 6b-4
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Potential Fungistatic and Parasiticidal Agents

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: None

Cooperating Units: Section on Mycology, LID-NIAID
Dr. Chester W. Emmons - NIAID No. 7C-4
Section on Chemotherapy, LPC-NIAID
Dr. George Luttermoser - NIAID No. 13C-2

Man Years (calendar year 1959)

Total:	12/12
Professional:	3/12
Other:	9/12

Project Description:

Objectives: Synthesis of new potential chemotherapeutic agents against fungal and parasitic infections.

Methods Employed: Antifungal agents: In a continuation of last year's project, additional thymol derivatives were synthesized by condensing various naphthoquinones and glycol chloroformates with p-amino-thymol in ether.

Since several antibiotic compounds have been isolated from green plants and identified as naphthoquinones having antifungal activity, a series of 50 naphthoquinone derivatives were synthesized by condensing various naphthoquinones with semicarbazides, thiosemicarbazides, aromatic amines, long chain aliphatic amines, sulfonamides, secondary amines and substitutedhydrazine.

The well-known tuberculostatic compound, 4, 4' diaminodiphenylsulfone also shows considerable antifungal activity. 20 derivatives of this compound were synthesized. The compounds were recrystallized

and analyzed for carbon, hydrogen and nitrogen. Toxicity studies at various dosage levels were performed in white mice.

Parasiticides: A series of 50 piperazine derivatives were synthesized by reacting anhydrous piperazine with carboxylic acids, sulfonic acids, isocyanates, isothiocyanates, acid chlorides and glycol chloroformates. Several antimony trichloride complexes of chlorotetracycline, oxytetracycline and tetracycline were also prepared for tests as potential chemotherapeutic agents against parasitic infections.

Major Findings: Several of the piperazine derivatives demonstrated considerable in vitro and in vivo activity against the pin worm and showed negligible toxicity for mice.

Significance to bio-medical research and the program of the Institute: The widespread clinical use of the antifungal and parasiticidal agents currently used is limited by their toxicity. Since mice have a high tolerance to the compounds synthesized, the drugs may be useful as potential chemotherapeutic agents against fungal and parasitic infections.

Proposed course of project: The compounds are being tested for their usefulness as possible chemotherapeutic agents in experimentally infected laboratory animals.

A by-product of the above study is the anti-tumor testing of the above compounds and also of compounds previously synthesized for possible tuberculostatic activity. Approximately 500 compounds have been sent to the Cancer Chemotherapy screening program. Seve[illegible] have passed the first recovery test in mice.

Control No. NIAID 61-B
1. Infectious
2. Medical & Physiological
Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Toxicity and Detoxification of the Antifungal Agents, Mycostatin and Ascosin

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 8/12
Professional: 2/12
Other: 6/12

Project Description:

Objectives: To find means of reducing the toxicity of these two highly active fungistatic agents.

Methods Employed: Concomitant administration by intraperitoneal injection in white mice of each drug with various potential detoxifying chemicals in attempts to reduce the toxicity which is always observed with these agents.

Major Findings: Mycostatin: Various amino acids, vitamins, organic solvents and aromatic sulfonic acids have been tested as adjuvants with this drug by intraperitoneal injection into white mice in both simultaneous and staggered treatments. Of the numerous adjuvants tested, nicotinic acid, pyridine-3-sulfonic acid and 6-thymolsulfonic acid, exhibited activity. Nicotinic acid gave optimal results in combination with toxic doses of 3 to 4 mg of mycostatin in either single or repeated intraperitoneal injections.

With Ascosin: The same adjuvant (nicotinic acid) that gave optimal results with mycostatin was also effective with ascosin, permitting 6 of 11 white mice to survive twice this lethal dose for 10 daily doses.

Part B not included

Serial No. NIAID 64-C
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on the Amino Acid Composition of Mycobacteria.

Principal Investigator: Dr. Benjamin Prescott

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 8/12
Professional: 2/12
Other: 6/12

Project Description:

Objectives: The non-pathogenic vole bacillus is currently in use as a vaccine against tuberculosis in England. Since BCG is admittedly an inadequate means of protection against tuberculosis but is the only existing extensively tried antigen, it seemed logical to determine if there was any similarity in structure of the vole bacillus and BCG. On the assumption that chemical structure determines immunological activity, the study may provide information as to the relationship of the chemical structure of each organism to antigenicity.

Methods Employed: Dried, heat killed organisms of both strains, grown under fixed growth conditions, were analyzed for protein and essential amino acids. A rapid quantitative method for amino acid determining using two-dimensional paper chromatography was developed.

Major Findings: The cellular protein from both species contained high concentrations of glutamic acid, 9.2%; leucine 7.8%; arginine 7.7% and alanine 7.2%, somewhat lower concentrations valine 4.2% and very low concentrations of histidine 0.2% and the sulfur containing amino acids. The concentration of lysine was about two-thirds that of arginine. Tryptophans was found in somewhat lower concentrations 2.6%

Part B not included

than lysine. The tryptophane was determined directly since the hydrolytic procedures used destroy most of this amino acid. All of the hydrolysates were extremely low in methionine. The ten essential amino acids were found in every hydrolysate. In these respects, the analytical data showed marked similarity between the vole bacillus and BCG.

Significance to bio-medical research and the program of the Institute: From the point of view of allergies and infectious diseases, it is of importance to determine the relationship between chemical composition of cells to infectivity, hypersensitivity and immunity.

Proposed course of project: Project terminated. Results to be published.

Serial No. NIAID 65
1. Infectious Diseases
2. Medical & Physiological Bacteriology

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigations of the type-specific immunity to Group A streptococci.

Principal Investigator: Dr. Fred S. Kantor

Other Investigators: Dr. Roger M. Cole

Cooperating Units: None

Man Years (calendar year 1959)
Total: 33/12
Professional: 18/12
Other: 15/12

Project Description:

Objectives: To improve methods of preparing antigenic streptococcal M protein and thus improve type-specific antibody response; to determine the relationship of enzyme-extracted M protein to material prepared by other methods; and to investigate possible mechanisms by which M protein affects virulence.

Methods Employed: A streptococcal cell-wall enzyme (lysin) is prepared from fresh phage lysates of Group C streptococci. This lysin is used to hydrolyze Group A streptococci cell walls and the resulting digest is fractionated by means of ammonium sulfate precipitation and ion exchange column chromatography. The "M" protein prepared by these methods is compared, with respect to antigenicity in rabbits, with "M" protein extracted by conventional means. For this purpose standard mouse protection tests, bactericidal properties, and precipitin analyses are used.

Major Findings: Streptococcal M protein preparations made by the method described is called "lysin M" to distinguish it from "acid M" prepared by classic methods of acid hydrolysis. Lysin M preparations were shown by reaction with appropriate antisera to contain, in addition to M protein, small amounts of group-specific "C" carbohydrate and the "T" protein. Such preparations failed to react with specific antiserum when the ionic strength of the solvent buffers was increased--thus differing from acid M which continued to react under such conditions. Lysin M was shown to be a good antigen: type-specific antibodies, as demonstrated by 4 tests (bactericidal,

Part B included: Yes

long chain growth, precipitin and mouse protection) developed in rabbits after intracutaneous injection of 1.5 mg. per animal. Following such injections, no local or systemic toxicity was noted. A manuscript describing this work in detail has been passed by the Editorial Committee, NIAID and portions are being revised as suggested by the Journal of Experimental Medicine for resubmission. (Kantor, F. S. & Cole, R. M. -- Preparation and antigenicity of M protein released from Group A, Type 1 streptococcal cell walls by phage-associated lysin.)

In the course of these studies, we noted that lysin M, as well as purified acid M and crude acid extracts from Group A streptococci contained a factor which precipitated mammalian plasmas and purified fibrinogens. Chicken plasma, even with added rabbit serum, did not react with this factor (which was called fibrinogen precipitating factor, or FPF). The reaction with mammalian plasma occurred in the presence of citrate, oxalate, or heparin. Diisopropylfluorophosphate did not inhibit FPF, nor did FPF hydrolyze tosyl-arginyl methyl ester synthetic substrate. The factor was found in acid extracts of 70 of 131 strains of 33 types of Group G strains: it was not found among 25 strains of streptococcal groups B,C,D,F, and K; nor viridans streptococci, staphylococci, and pneumococci. The failure of acid extracts on some M-typable strains to demonstrate FPF may be due to capsular material, because added hyaluronic acid during extraction inhibited FPF without interfering with M-typing. In addition, purification of M from initially FPF-negative extracts resulted in positive reactions. Treatment of FPF-positive M extracts with pepsin and trypsin destroyed both serologic typability and the reaction with fibrinogen, and T extracts of FPF-positive strains were FPF-negative. Type specific antiserum and fibrinogen each absorbed both serologic reactivity and FPF from solutions of M protein. Thus M protein and FPF appear inseparable. The properties of M protein and staphylococcal coagulase as related to virulence appear somewhat analogous.

Significance to bio-medical research and the program of the Institute: Immunity to Group A streptococci, in humans as well as animals, appears to be type-specific and dependent therefore on antibody to M protein. The poor antigenicity of purified M protein as conventionally prepared as well as reactions to cruder preparations discouraged studies of active immunization of humans. Lysin M, although containing traces of other streptococcal antigens, is nevertheless a good antigen without obvious toxicity in animals, and thus offers promise for restricted groups of humans or in special situations where streptococcal immunity would be feasible and of importance.

The demonstration of a fibrinogen precipitating activity in M protein preparations appears to be one of the first demonstrations of a biologic activity of an intracellular bacterial substance associated with virulence. Speculation as to the role of FPF in preventing phagocytosis, inducing fibrinoid lesions, and the like, are of interest and further investigations of such possibilities are in progress.

Proposed course of project: Continued investigations of the role of M protein (or FPF) in various biologic and pathogenic activities of Group A streptococci are planned. Dr. Fred S. Kantor is now at Grace-New Haven Hospital and Yale University, and the studies are being made with Dr. Jerome W. Hahn (See Project Serial No. NIAID 65-A).

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Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Kantor, F. S. and Cole, R. M. A fibrinogen precipitating factor (FPF) of Group A streptococci. Proc. Soc. Exp. Biol. Med., 102:146-150, 1959.

1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of M protein and virulence

Principal Investigator: Dr. Jerome W. Hahn

Other Investigators: Dr. Roger M. Cole

Cooperating Units: None

Man Years (calendar year 1959)
Total: 19/12
Professional: 12/12
Other: 7/12

Project Description:

Objectives: To determine the means by which M protein is responsible for the virulence of typable streptococci of Group A.

Methods Employed: Standard bacteriological, biochemical, immunological techniques.

Major Findings: Numerous vaguely defined areas, presumptively related to M protein, are being investigated. These include the clumping of streptococci in plasma and fibrinogen solutions; protection against phagocytosis by M protein; pathologic effects of M protein in vivo and in leucocytes in presence of antibody; transformation reactions between M-types of Group A streptococci; etc. No reportable results at present, since Dr. Hahn has been on duty only since the beginning of the fiscal year.

Significance to the program of the Institute: (new project)

Proposed course of project: Continuation

Part B not included

1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Phage Transduction in Staphylococci

Principal Investigator: Dr. Roger M. Cole

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 22/12
Professional: 1/12
Other: 21/12

Project Description:

Objectives: To confirm and extend preliminary reports of phage-mediated genetic transfer in staphylococci. Investigation of the role and effects of double and multiple lysogeny in genetic transfer will be a concurrent study.

Methods Employed: Using selected markers, e.g. streptomycin resistance, novobiocin resistance, and various carbohydrate fermentations of donor strains, phage lysates of the above are exposed to potential recipient strains with reciprocal markers and the resultant suspensions are plated onto media suitable for detecting any transduced positives. (Methods of Stocker, Lederberg, Zinder, Baron and others).

It is desirable to investigate some of the environmental factors which may affect transduction, ie., (1) media changes (2) presence of extracellular DNA slime layer in possible recipients (3) elaboration of extracellular DNase by possible recipients (4) lysogenic phage in donor strains.

Attempts will be made to transduce some of the "virulence" properties such as toxin production and coagulase of pathogenic strains to innocuous staphylococci.

Part B not included.

Major Findings: A staphylococcal strain (#610, phage pattern 52/52A/80/42B/81) was made streptomycin and novobiocin resistant. Phages 52, 52A, and 80, propagated in the drug-resistant strain to high titer were then mixed with a recipient staphylococcal strain (#659, phage pattern 42B/81) and plated onto antibiotic plates after incubation. Antibiotic-resistant colonies did not appear in numbers greater than in the control platings without phage. Also, a high multiplicity of infection was required because of expected low rates of transduction: as a result, many recipient cells of strain #659 were lysed and it was later shown that this strain was indeed sensitive to undiluted stocks of phages 52, 52A, and 80.

In an attempt to use markers other than antibiotic resistance, lactose-negative and mannitol-negative fermentation mutants of strain #659 were obtained by ultra-violet irradiation. Attempted transduction by phage of lactose and mannitol-positive markers to #659 from strain #610 gave inconclusive results.

It was found that the 5-pattern strains (52, 52A, 80, 42B, 81) could be easily lysogenized with free phage from 2-pattern strains (42B, 81). Using the lysogenizing phage as a possible agent, transductions involving antibiotic-resistance and fermentation markers were again tried. The results were inconclusive.

Significance to bio-medical research and the program of the Institute: Genetic transfer by DNA and phage transduction has been profitably investigated in several bacterial genera but not extensively in staphylococci. Published reports in work of this type with staph probably number less than half a dozen to date. Since staphylococci have assumed increasing importance in respect to its troublesome transmissible characteristics, its ubiquitousness and antibiotic resistance, investigations leading toward elucidation of any segment of its basic genetic nature would be helpful.

Proposed course of project: Terminated because of transfer of Mr. Ikari and Miss Moore to other areas of NIH.

Serial No. NIAID-5
1. Infectious Diseases
2. Medical & Physiological Bacteriology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of Upper Respiratory Tract Bacterial Flora of Young Children

Principal Investigator: Dr. Roger M. Cole

Other Investigators: Edythe J. Rose

Cooperating Units: None

Man Years (calendar year 1959)
Total: 8/12
Professional: 3/12
Other: 5/12

Project Description:

Objectives: To observe the occurrence, both endemic and epidemic, in sickness and health, of several pathogenic bacteria in the upper respiratory tracts of children.

Methods Employed: Direct throat swabs are taken routinely once weekly from a nursery population (up to three years of age), as well as at times of admission, illness, and discharge. Platings are made on appropriate media, incubated, and relative numbers of the various bacteria recorded. Colonies of beta-hemolytic streptococci, Hemophilus spp., pneumococci, and beta-hemolytic staphylococci are identified and picked for purification.

Further identification is accomplished biochemically and by specific antisera for streptococci, pneumococci, and Hemophilus influenzae. Staphylococci are identified by phage typing. Bacterial strains isolated are preserved by lyophilization and added to the collection for future reference. A record of each preserved strain is kept on keysorter punch made cards, and a similar annotated individual card is kept for each culture result on each child. Results are being tabulated and analyzed by various attributes.

Part B. Not included

Major Findings: Laboratory work on this project was discontinued on June 30, 1959. In this past year, in conjunction with the Epidemiology and Virus and Rickettsial Sections, LID, preliminary analyses of the accumulated data have been made. The results were presented in preliminary fashion at the annual meeting of the American Public Health Association in October 1959. (Bell, J. A., Huebner, R. J., Rowe, W. P., Rosen, L., Cole, R. M., Mastrota, F. M., Floyd, T., and Chanock, R. M. -- Illness and microbial experiences of nursery children at Junior Village, a District of Columbia welfare institution. II. The plan of study and general epidemiologic observations.)

In the first 3 years of study (156 weeks), bacteriologic throat specimens were taken during the latter 83 weeks: the total child-weeks thus sampled was 4598. In this time period, 2313 child-infections with specified bacteria as determined by isolation occurred: 2088 of these were infections with presumptive pathogens from the throat and the balance were enteric bacteria isolated from anal swabs by another laboratory. Of the 2088 child-infections of the throat, 260 were caused by beta-hemolytic streptococci, 1043 by Hemophilus spp., 515 by staphylococci, and 270 by pneumococci.

Most of the streptococcal infections were due to members of serologic group A (194/260 = 74.6%), in which were included 8 different serologic types; smaller numbers of infections with Groups G, C, B, and D, in that order, made up the balance. Infections with each serologic type of Group A occurred in discrete outbreaks: only these infections, as contrasted with those due to the other throat bacteria isolated, showed significant temporal relations to the occurrence of clinically undifferentiated definite febrile illness.

Approximately 40% of child-infections with species of Hemophilus were caused by H. influenzae: half of these were due to encapsulated, type-specific strains, of which again approximately half were of Type B. Non-type-specific H. influenzae, H. parainfluenzae, H. hemolyticus, H. parahemolyticus and unidentified Hemophilus spp. caused 80% of all Hemophilus infections.

Of 515 staphylococcal infections, 326 (63%) were caused by hemolytic, coagulase-positive strains and were phage-typed: the majority of these (76%) were sensitive to phage 81--usually, however, in various combinations with other phages. The most common single phage pattern was 42B/81.

Thirteen serologic types of Diplococcus pneumoniae, found in 50% of the total infections were found among 270 pneumococcal child-infections. A few other pneumococci failed to type with the sera available, and the remainder could not be tested serologically. Types 23 and 19 were isolated most commonly.

The highest mean weekly new infection rate for bacteria was 5.3% for typed pneumococci, and was significantly greater in children less than 16 months of age than in older children. In contrast, new infections with typed Group A streptococci (overall rate 2.7% were significantly higher in children over 16 months old. Type B H. influenzae showed no significant difference by age; and although staphylococci sensitive to phage 81 were found in new infections most often in the younger age group, the significance was dubious. By race or sex, no significant differences for new infection rates by any of these bacteria were found.

Significance to bio-medical research and the program of the Institute: The frequency of undifferentiated acute respiratory infections and fevers of unknown etiology is well recognized: that most of these may occur in children has recently been emphasized. Although the number of presumptive new etiologic agents has grown steadily, there is still a large residue of such infections for which the cause is yet uncertain. In the continued search for new causative agents, it is most pertinent to include testing for known agents--including bacteria--to establish some sort of baseline of their relation to undifferentiated disease, their prevalence in non-ill children, and their role, if any, in pre-disposition to other agents.

Our studies to date, although showing the common occurrence in throats of young institutionalized children of several bacteria of presumptive pathogenicity, have failed to show, except for typable strains of Group A streptococci, a relation of these organisms to disease in the population under study. Further study in collaboration with the Epidemiology and Virus and Rickettsial Sections should elaborate on the general prevalence of these bacteria, their duration in the individual hosts, their relation to antibiotic administration and prophylaxis, and their role, if any, in predisposing to acquisition of other agents of disease.

Proposed course of project: Continued epidemiologic and statistical study of accumulated data, especially detailed study of each genus of bacteria isolated. Correlation with results of virus studies.

Journal No. NIAID 66
1. Infectious Dis...
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epidemiologic Studies of Illnesses and Microbial Experience of Junior Village Nursery Children

Principal Investigator: Dr. Joseph A. Bell

Other Investigators: Dr. Francis M. Mastrota and Dr. Robert J. Huebner

Cooperating Units: Virus and Rickettsial Diseases Section, LID, (Dr. Chanock, Dr. Johnson), Epidemiology Laboratory Unit, (Dr. Rosen), Respiratory Bacteria Unit, (Dr. Cole), Oncolytic and Oncogenic Virus Unit, (Dr. Rowe), Naval Medical Research Institute, (Dr. Floyd).

Man Years (Calendar year 1959):
Total: 111/12
Professional: 27/12
Other: 84/12

Project Description:

This is a long term, very intensive study of the illness and microbial experiences of nursery children at Junior Village, a District of Columbia Welfare Institution. It began in July 1955 and provides research material for many projects, e.g., 66A, 66B, 67.

(A) Basic Objectives: The general objective is to maintain under observation a population group suitable for epidemiologic study of occurrence of infection and disease and host-parasite-disease-relationships as they occur naturally in this group and as they can be altered by chemo prophylaxis and new vaccines. One of the chief interim objectives is the development of epidemiologic, clinical and laboratory tools and methods for studying infectious diseases. A concerted effort is being made to find new microbiologic agents which cause disease, methods of identification of these agents, modes of spread and methods for prevention and treatment of acute illnesses, particularly respiratory illnesses. The study is designed to shed light on the nature and scope of studies which should be pursued in further search for methods of control of acute infectious diseases particularly, the large mass of acute respiratory diseases, including the common cold.

More specific objectives are noted in projects, 66A, 66B, 67.

Part B None

Methods Employed and Patient Material:

The study children are located in Southwest Washington, D. C. The daily population is now close to 100 white and negro babies six to 35 months of age who are in residence in Eisenhower Cottage for domiciliary care. The population turnover is great. The mean duration of residence is approximately 17 weeks per child. Children with illnesses are studied either in their domicile or the infirmary at Junior Village, or the Clinical Center at N.I.H., or at the D.C. General Hospital, depending upon the severity of illness and study interest.

A full-time pediatrician, three nurses, and four nurses' assistants maintain constant medical surveillance of the children, record rectal temperatures twice every day, collect specimens for laboratory study and prepare clinical records on each child each day, regardless of whether or not ill. Both throat and anal specimens for virus study are collected on admission, and three times weekly from every child, and otherwise when indicated. Blood specimens are collected on admission, on discharge, six weeks after admission, every three months during residence, and under special circumstances.

Major Findings:

Epidemiologic methods have been devised for recognition of febrile departure from normal health and illnesses have been classified as (A) questionable fever, (B) definite fever with clinical findings (FUO) and (C) definite illnesses, i.e., fevers associated with clinical findings.

An astounding number of children suffered acute illnesses, -- 11% per week had onset of questionable fever (A), 15% had FUO (B) and 10% per week had definite febrile illness (C). Thus, during an average week, 1/4 of the babies had onset of a definite acute undifferentiated illness, i.e., excluding measles and chickenpox. These illnesses had little seasonal variation and the attack rates were similar by age, sex and race. The attack rates decreased with duration of residence in the nursery group indicating the development of immunity to disease.

During the three-year observation period an astounding number of microbial infections were found. A total of 1933 child infections with 55 different virus types were found and a total of 2313 child infections with bacteria were delineated, 718 of the latter were presumably pathogenic bacteria. During periods of adequate routine culturing for specific agents it is crudely estimated that 20% - 35% of the children acquired a new virus or a new bacterial (presumably pathogenic) infection each week. The new infection rates of the various agents were similar by sex, slightly higher in the white than in the negro race, and generally higher among the younger babies with the exception of myxovirus infections and group A Beta Hemolytic Streptococci infections. The enteroviruses occurred predominantly from July to December, whereas the adenoviruses occurred predominantly from January to June, but the seasonal differences were not as marked as anticipated. The enteroviruses and Type 3 and 5 adenoviruses and typed Group A Streptococci infections occurred in sharp outbreaks as contrasted with other

infections which occurred more endemically. In epidemic outbreaks of infection practically all susceptible children present were infected within two to three incubation period cycles and then the infection persisted in the institution often for many months by successive infection of newly admitted children.

Although epidemiologic methods are still being sought to delineate etiologic relationships, the findings to date show that most of these virus and bacterial infections cause no detectable illness but crude estimates indicate that perhaps some 45% of the more severe illnesses are due to known agents.

Significance to Program of the Institute:

This project provides a source of specimens for finding new causes of illness, for studying their etiologic significance and for testing methods of control. It is significant that long term Junior Village studies of host-parasite-disease-relationships are being carried out on the bulk of diseases which commonly cause misery and absenteeism from schools and industry. The studies are of such a nature that they are not likely to be conducted by other than a government group. The studies are progressing meticulously and fairly satisfactorily to the investigators.

Proposed Course of Project:

Clinical epidemiological observations are being continued together with collection of throat and anal specimens three times weekly for virus study. Throat specimens are being studied for myxoviruses and other new viruses. A random sample of the anal swab specimens are being tested for enteroviruses so as to enable selection of specimens for intensive study. Various new vaccines are being given preliminary trial in this population group. It is planned to continue the study for some two years or as long as it continues profitable.

1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epidemiologic Studies of Host Parasite Disease Relationships

Principal Investigator: Dr. Joseph A. Bell

Other Investigators: Dr. Francis M. Mastrota and Dr. Robert J. Huebner

Cooperating Units: Virus and Rickettsial Diseases Section, LID, (Dr. Chanock, Dr. Johnson), Epidemiology Laboratory Unit, (Dr. Rosen), Respiratory Bacteria Unit, (Dr. Cole), Oncolytic and Oncogenic Virus Unit, (Dr. Rowe), Naval Medical Research Institute, (Dr. Floyd).

Man Years (Calendar year 1959):
Total: 39/12
Professional: 9/12
Other: 30/12

Project Description:

(1) Objectives: To delineate which of the many microbial agents are causing acute illness in Junior Village nursery and describe clinical and epidemiological nature of etiologic entities found.

(2) Patient Material: The Junior Village nursery studies are described in project number 66. Epidemiologic methods are being devised to determine which of the 53 typed viruses and the many typed bacteria, which have been isolated, were causing illness.

The high frequency of illness occurrence (mean weekly definite illness attack rates of 25%) and the even higher frequency of new and overlapping infections with potentially pathogenic microbial agents presents an extremely complex epidemiological problem to establish specific etiologic relationships. One of the chief problems is serological determination of susceptibility and immunity to the many specific infections, or illness attributable thereto; with the limited amounts of serums available. To guide the most profitable use of these serums and for other purposes preliminary analyses of temporal relationships between onset of specific infection and onset of acute undifferentiated illnesses have been completed and top priority for use of available serums can be established on the basis of resolving the etiologic relationship of the more serious illnesses, study of the many newly

Part B Included Yes

discovered viruses and study of new vaccines. Within these limits the meticulous and time consuming analysis of specific etiologic relationships is in progress.

Major Findings:

The preliminary analyses of temporal relations between onset of specific infection and onset of acute undifferentiated afebrile illness using both horizontal and cross sectional controls have shown no evidence that the following agents were causing illness in the nursery babies: Adenovirus 2; Polio 3; Coxsackie B 5; ECHO 7, 8, 11, 12, 13, 14, 18, 19, 20, 25, and JV 5; nontypable Group A Streptococci; Hemophilus Influenza B; Pneumonia Type 19, 23; Staphylococci having Phage Type 81 in their antigenic pattern; Enteropathogenic coli of various types, and alkalescens dispar. Infection with the following agents were significantly associated with the occurrence of acute, febrile, undifferentiated illness; Adenovirus 1, 3, and 5; Influenza virus Asian; parainfluenza virus 1 and 3; Poliovirus 2, Coxsackie virus B 3; Group A Streptococci types 4, 12, 23, and 1, 2, and 5; and Shigella Sonnei. In general, it is crudely estimated that some 45% of the more severe illnesses of the nursery group may be accounted for by the above agents and that measles and adenovirus infections account for the bulk of the more severe illness.

Significance to Program of the Institute:

Although the study is deliberately limited to a group of young children who have a high proportion of susceptibles to various microbial infections, it shows some of the potentialities of such infections in older susceptible persons. This project represents a systematic approach to determining the various etiologies of acute febrile illness so as to guide research on development of methods for control.

Proposed Course of Project:

It is planned to continue the study for two years or as long as continued collection of data and data analyses appear profitable.

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R.M., Vargosko, A., Luckey, A., Cook, M.K., Kapikian, A.Z., Reichelderfer, T.E., and Parrott, R.H.

"Association of Hemadsorption Viruses with Respiratory Illness in Childhood"

J.Amer. Med. Assn. Vol. 169:548-553 (Feb. 1959)

Awards:

None

Honors:

None

Serial No. NIAID 66-B
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epidemiologic Studies of New Vaccines and Chemoprophylactic Agents

Principal Investigator: Dr. Joseph A. Bell

Other Investigators: Dr. Francis H. Mastrota, Dr. Albert Z. Kapikian, Dr. Robert J. Huebner

Cooperating Units: Virus and Rickettsial Diseases Section, LID, (Dr. Chanock, Dr. Johnson)
Epidemiology Laboratory Unit, (Dr. Rosen)
Respiratory Bacteria Unit, (Dr. Cole)
Oncolytic and Oncogenic Virus Unit, (Dr. Rowe)
Naval Medical Research Institute (Dr. Floyd)

Man Years (Calendar Year 1959):
Total: 9/12
Professional: 3/12
Other: 6/12

Project Description:

Objectives: (A) To design and have prepared monovalent and multipolyvalent vaccines with inactivated viruses which have shown evidence of producing important illnesses. (B) To carry out preliminary trials of such vaccines for determination of antigenicity and untoward reactions. (C) To observe whether such vaccines substantially reduce the occurrence of infection or illnesses in the Junior Village Institution.

Patient Material: The Junior Village nursery studies are described in project numbers 66, and 66-A. The rather intense daily clinical observation permits an evaluation of vaccine induced reactions. The vaccines which have been used are: (1) Adenovirus types 1, 2, 3, and 5; (2) Adenovirus types 3, 4, and 7; (3) Coxsackie B virus types 1, 2, 3, 4, and 5; (4) Rubeola virus; (5) Respiratory vaccine containing 12 virus strains, namely, monkey kidney cell grown influenza A1, A2, and B viruses; adenovirus types 1, 3, 4, 5, 7, parainfluenza virus types 1 and 3, and CA viruses. In addition, a daily oral dose of 300,000 units of benzathine Penicillin has been given to a 20% random sample

Part B None

of all children. The vaccines are given at time of admission, with the second dose three weeks later and a third dose at three months. The vaccines are given in one ml doses to preselected random samples of all children admitted to the Junior Village nursery group. Blood sera for antibody studies is collected on admission, at six weeks, and every three months thereafter and at discharge.

Major Findings:

A rubeola outbreak occurred in February shortly after start of the measles vaccines. Only 11 children had received a dose of rubeola vaccine prior to the outbreak. Of these, only two developed measles whereas more than 50% of the children not receiving the vaccine were attacked. This is a strikingly significant difference indicating vaccine protection but the numbers are too small to warrant publication. Preliminary analyses of the adenovirus vaccines No. 1 and 2 (above) indicate no protection against infection but analyses must await serologic studies to determine the susceptibility and immunity. These vaccines were discontinued in July and the 12 strain multipolyvalent vaccine initiated. No analyses have been made on the effects of the Coxsackie B virus vaccine.

The trial of penicillin prophylaxis was discontinued in July when preliminary crude analyses indicated no differences in the occurrence of illness among the test and control groups. Although children given prophylactic penicillin did not shed streptococci infections, the occurrence of viral infections was not altered. Further analyses pinpointed to streptococcal epidemics are necessary to confirm or establish the value of the penicillin prophylaxis and the possible symbiotic relation of streptococcal and viral infections.

Significance to Program of the Institute:

It is obvious that the many acute febrile respiratory diseases and fevers of undetermined origin which are a major public health problem are caused by a great multiplicity of etiologic agents. To prevent or ameliorate these illnesses by vaccine prophylaxis requires a multipolyvalent vaccine in as much as no one of these known agents are producing a very large proportion of these diseases. It seems timely to initiate controlled epidemiologic studies in the use of multivalent vaccines and their possible enhancement or interference with each other.

Proposed Course of Project:

It is planned to continue the study for two years or as long as continued collection of data and data analyses appear profitable.

1. Infectious
2. Epidemiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Enteroviruses found in the Human Alimentary Tract

Principal Investigator: Dr. Leon Rosen

Other Investigators: Dr. Joseph A. Bell, Dr. Francis M. Mastrota, Dr. Robert J. Huebner, Mrs. Janet F. Hovis

Cooperating Units: D. C. Welfare Department

Man Years (Calendar Year 1959):
Total: 72/12
Professional: 7/12
Other: 65/12

Project Description:

A. Objectives: To determine the role in human biology of the enteroviruses and other viruses found in the alimentary tract of man.

Methods Employed: The principal population group under study is an orphanage nursery in the District of Columbia, whose average population is approximately 60 children between the ages of 6 months and 3 years. The turnover of this population is relatively rapid, and as a result, there is a great deal of illness throughout the year. (See Project No. 66 Dr. Bell's description of Junior Village.)

Rectal temperatures of each child are obtained twice a day, and the entire group is observed and provided medical care by a full time pediatrician.

Throat and anal swabs are obtained routinely once a week from all the children, and additional specimens are obtained when indicated. Serum specimens are obtained on admission and on discharge and at various intervals between.

Part B. Included Yes

The throat and anal specimens are tested in various tissue culture systems, and where indicated, in animals. The sera are used for antibody studies.

Finally, laboratory data are correlated with clinical, bacteriological, and epidemiologic information in an effort to determine virus disease relationships.

In addition, selected outbreaks in other populations of illness suspected to be due to the viruses under study are investigated to provide kinds of data not available from the study of the limited age group present in the nursery group.

Cooperative studies are also carried out to determine if viruses similar to human enteroviruses which occur in domestic animals are capable of infecting man and if human alimentary tract viruses can infect domestic animals.

Major Findings:

The typing of enteroviruses and adenoviruses isolated from the nursery population during the year from July 1958 to July 1959 was largely completed. Typing techniques which were developed last year were employed for rapid identification of these agents.

An epidemic of Type 16 Adenovirus was picked up during the winter months of this period. All isolates were recovered from anal specimens, further substantiating a previous finding that these lesser known Adenos are more commonly found in the lower intestinal tract.

The outbreaks of two ECHO 10 (now known as Reovirus) agents were analyzed and evaluated. Type 1 Reovirus occurred in early 1957, infecting at least 43 of 73 children then present. An association of borderline significance was noted between the onset of reovirus type 1 infections and the occurrence of mild febrile illness.

Two outbreaks of reovirus type 3 involving at least a total of 46 children occurred in 1955 and 1957. Hemagglutination-inhibition antibody responses were quite type-specific and occurred in each of the children tested from whom the virus was recovered, as well as in a number of other children present at the same time. Antibodies to Type 3 Reovirus were found to be very prevalent among children in the United States, Panama, the South Pacific, as well as among a number of species of domestic animals and laboratory primates.

Significance to Program of the Institute:

This project represents a unique effort to acquire a more complete understanding of the virologic experiences and the disease consequences of such experiences in young children.

Proposed Course of Project:

The 1958-59 portion of this study will be continued with emphasis on the serologic and clinical aspects of the infections which have occurred

Part B:

Publications:

Rosen, L., Hovis, J.F., Mastrota, F.M., Bell, J.A., Huebner, R.J.

"An Outbreak of Infection with Type 1 Reovirus Among Children in an Institution"

Rosen, L., Hovis, J.F., Mastrota, F.M., Bell, J.A., Huebner, R.J.

"Observations on a Newly Recognized Virus (Abney) of the Reovirus Family."

Rosen, L.

"Serologic Grouping of Reoviruses by Hemagglutination-Inhibition"

Rosen, L.

"A Hemagglutination-Inhibition Technique for Typing Adenoviruses"

N.B. All above listed publications have been accepted for publication in the American Journal of Hygiene.

Honors: None

Awards: None

Record No. [illegible]
1. Infectious [illegible]
2. Epidemiology
3. Bethesda, [illegible] and

FMC-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of enteroviruses prevalent in man and animals on the Island of Tahiti. Study of an outbreak of Eosinophilic Meningitis in Tahiti.

Principal Investigator: Dr. Leon Rosen

Other Investigators: None

Man Years (Calendar year 1959):
Total: 12/12
Professional: 4/12
Other: 8/12

Project Description:

Basic Objectives: (1) A study of these enteroviruses in a unique epidemiologic situation in order to broaden our knowledge of the biology and natural history of these viruses. (2) An effort to determine the etiology of the Eosinophilic Meningitis occurring in Tahiti.

Methods Employed: Sera have been collected on several herds of cattle and a group of dogs and cats and tested against the Reovirus types 1, 2, and 3.

Anal swabs have been taken from young children attending a well baby clinic in Tahiti and have been checked for enteroviruses.

A series of serum specimens and virus specimens from persons with Eosinophilic Meningitis have been checked out to eliminate all the known possible agents of this disease.

Major Findings:

It was shown that cows, dogs, and cats in Tahiti show serological evidence of infection with T 1, 2, and 3, Reoviruses. T 3 Reovirus appears most prevalent just as it does among herds in Maryland.

ECHO 11, Adeno Types 1, 2 and 16, have been isolated, so far, from young children attending a well baby clinic in Tahiti.

To date, no known agent has been implicated in the current outbreak of Eosinophilic Meningitis on Tahiti.

Part B None

Significance to Program of the Institute:

An island such as Tahiti which is somewhat isolated from the major world populations affords an excellent opportunity for study of the epidemiology of viruses.

The determination of the etiology of Eosinophilic Meningitis would be of obvious value in order that appropriate preventive measures could be developed.

Proposed Course of Project:

The study on Eosinophilic Meningitis will be continued in an effort to determine its etiology. Constant observation for evidence of other outbreaks which might give worthwhile information will be maintained.

Field work in Tahiti will close early in 1960. However, sera epidemiology of viral infections of natives living in this unique environment will be continued at the Bethesda laboratories.

Serial No. NIAID 67-B
1. Infectious Diseases
2. Epidemiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of enterovirus and reovirus infections of domestic animals and natural and experimental infection of cattle with human types of reoviruses.

Principal Investigators: Dr. Leon Rosen, Dr. F.R. Abinanti

Other Investigators: None

Cooperating Units: Dairy Husbandry Department, College of Agriculture, University of Maryland.
Maryland Home of Correction, Jessup Maryland

Man Years: (Calendar year 1959)
Total: 12/12
Professional: 1/12
Other: 11/12

Project Description:

Objectives: (1) To recover viral agents from the gastrointestinal tract of cattle and to study their clinical importance and epizootiology; (2) To investigate the biological properties and natural histories of these viruses and particularly their similarity to viruses of man.

Methods Employed: Sequential monthly specimens of feces and blood have been taken on all calves born within the last year on three dairy farms. A total of 138 calves have been included in the study to date. The fecal specimens are inoculated onto bovine and monkey kidney tissue cultures. The viral agents that can be identified easily by HI or CF techniques are tentatively classified and pathogenisis studies on certain of the viruses have been done in susceptible calves. Other unidentified agents are frozen for future reference.

Major Findings: Reoviruses (ECHO 10), principally type 3, have been recovered on numerous occasions from these calves. The type 3 strains appear to be antigenically indistinguishable from the prototype recovered from man. Calves have been infected with this virus by means of aerosol inoculation. No evidence of clinical illness was observed, but the virus was excreted, infecting other calves, and specific antibody was produced.

An apparently new hemadsorbing virus was recovered from bovine intestinal tracts on numerous occasions on all three farms. At least

Part B. Included Yes

two of these are known to be similar on the basis of hemagglutination-inhibition. The character of hemadsorption appears to be different from any thus far observed in other viruses.

Several hundred isolations of enteroviruses have thus far been made from these calves; their importance in bovine disease and their relationships to similar human agents are under study.

Significance to the Program of the Institute:

A longitudinal study of enterovirus infections of animals under close observation at all times should furnish information on the clinical significance, spread, and seasonal distribution of various enteroviruses. Further, as in the case of the reoviruses, it will provide opportunities for developing information on the antigenic relationship of these viruses to those of man and furnish models if not actual source materials of human infections.

Proposed Course of the Study:

The field phase of the study will be completed in March, 1960. A thorough study of the viral serotypes obtained will be done; of special interest will be their relationships to human agents. With this information, serological studies will also be done to help delineate their clinical significance and possible mode of spread.

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Rosen., L., and Abinanti, F.R.:

"Natural and experimental infections of cattle with human types of reoviruses."

American Journal of Hygiene (in press - March, 1960)

Honors and Awards relating to this project:

None

Serial No. NIAID - 68
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of Tumor Viruses in Nature.

Principal Investigators: Dr. Robert J. Huebner, and Dr. Wallace P. Rowe

Other Investigators: Dr. Janet W. Hartley, Mr. Richard Peterson (Biologist, McGill University) Mr. John Estes, Mr. William T. Lane, Mr. Horace C. Turner, Dr. David O. White, Dr. Alexander Kohn (Guest Worker), Dr. Francis R. Abinanti, and Dr. Connie Yunker (Entomologist, University of Maryland).

Cooperating Units: New York City Health Department.

Man Years (calendar year 1959)
Total: 46/12
Professional: 12/12
Other: 36/12

Project Description:

Objectives: This new and major project is aimed at developing information for the first time on the natural history of tumor viruses, i.e., the natural prevalence, the methods of spread, the clinical consequences and the possible means for prevention of such infections.

Methods Employed: Several animal tumor viruses can now be grown and studied rather easily in laboratory and domestic animal systems. These include polyoma, avian leukosis, Rous sarcoma and papillomas. Of primary importance (which makes these studies possible) was the development of virological and serological techniques for quantitatively assessing (for the first time) the occurrence of these viruses in their natural hosts.

Part B included: Yes /X/ No /__/

Field studies [illegible] in order to study [illegible] in nature, this project required that we be [illegible] and when the answers to our questions can best be found by observation of natural phenomena. The natural hosts in which the tumor viruses found to exist must be observed by trained zoologists and the contributions of both general and specific environments assessed. Specimens for virological and serological study are collected and tested by various usual and newly developed virus techniques (see other projects, Rowe, Hartley, 71, 71A, 72).

Major Findings: The field studies were initiated in July 1959 (F.Y. 1960). Emphasis was placed primarily on the occurrence of polyoma infection in naturally occurring mice, although some effort was also successfully devoted to the natural distribution in certain chicken populations, of a virus thought by Burmester and Sharpless to be avian lymphomatosis.

The polyoma field study was set up in New York and certain rural areas. A preliminary survey of wild mouse serums to locate areas of infection, at first raised serious questions about whether polyoma was actually a natural infection of mice. Approximately 500 sera taken in 43 areas of several burroughs of New York were all negative. However, subsequent surveys in densely populated low economic areas of Harlem revealed not only much denser mouse populations than observed elsewhere, but also clear evidence of infection with polyoma virus. All available means of detection (HI, CF, neutralization, MAP tests and virus isolation in tissue culture) confirmed widespread infection in several Harlem mouse populations. The field study had to be abandoned in November, 1959, for lack of travel funds. Much laboratory work remains to be completed and analyzed. The following preliminary observations can be made:

(1) It is apparent that the natural history of an established tumor virus can be studied (independently of clinical manifestations) as easily or more easily than most ordinary respiratory or enteric viruses.

(2) Over 2000 mice were surveyed - 25-50% of some populations were found serologically positive for polyoma.

(3) Mice of all ages and sizes were positive.

(4) Virus was isolated from tissues and urine of 25% of the serologically positive mice tested.

(5) Analysis of two isolates from wild mice revealed high tumorigenic activity.

(6) Arthropods and insects so far studied (A. sanguineus and L. Bacoti) and various species of roaches appeared unimportant as reservoirs of virus but frequent mechanical transfers of virus could easily occur.

(7) Extracts of materials (bedding) from wild mouse nests in serologically positive areas yielded virus in 6 of 25 specimens.

(8) Release and retrapping of serologically negative wild mice in the natural nidus of infection resulted in at least five instances of conversion to positive serology. Virus was isolated from one of these.

(9) Tests of 60 humans living in apartments heavily infested with positive mice were considered negative for polyoma antibodies despite a few low level reactions in the HI test.

(10) Rats, mostly rattus rattus, were uniformly negative, even when trapped in buildings containing numerous "positive" mice.

(11) Other mouse viruses were isolated for the first time from wild mice. Mouse salivary gland virus was found in nearly 80% of New York City wild mice successively tested for this agent; it was also found commonly in rural mice. Reovirus type 2 was also recently isolated from New York City mice. Both these apparently latent agents were found in mice also carrying a demonstrably latent infection with polyoma.

(12) Because of intermittent spontaneous infections of mice in the Bethesda laboratory, efforts at primary isolations of polyoma from wild mice and others had to be carried out in a field laboratory in New York City where work with laboratory strains could not possibly influence the outcome of experiments.

This latter unanticipated necessity added additional expense to the project, helped exhaust travel funds, which led to the cessation of this study for the balance of F.Y. 1960.

Significance to the Program for the Institute: This type of study is unique in the annals of tumor virus research, provides a pattern for future similar efforts, and has excited considerable interest amongst cancer investigators everywhere. Perhaps what is most needed in tumor virus research is the demonstration that cancer viruses need not be extraordinary -- a special case in virology requiring radically new and as yet undiscovered procedures. We have been able to disclose nothing really mysterious about the natural history of polyoma -- it is clear that infection is not universal or inevitable -- and that under many conditions a cancer virus and its natural host can be kept apart, even by nature.

No polyoma tumors were observed in nearly 500 naturally infected mice (even though the virus strains isolated were tumorigenic)

and none were expected. Like many, if not most common respiratory and enteric viruses of man and animals, nearly all of the pathogenic activity of this tumor virus in nature is inapparent; again emphasizing the similarities instead of differences between this tumor virus and many other well-understood agents, and suggesting that given a specific etiologic agent of cancer, the natural history of which is known, quite conventional and well tested preventive medicine procedures may be quite adequate for the prevention or control of tumor virus activity.

Proposed Course of the Project: This field study is now suspended. We intend to resume field studies of the natural history of polyoma and to develop similar natural history studies on the other tumor viruses for which suitable laboratory methods are available whenever realistic support for field work on tumor viruses can be obtained. It should be repeated that natural history studies of animal tumor viruses represent the primary research interest of at least five professional investigators in the LID Virus Section.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this Project:

None - a new project.

Honors and Awards relating to this project:

Detailed reports on the unpublished work reviewed in this project have been requested for presentation at numerous meetings:

The International Symposium on Cancer, East Berlin, December 6, 1959.

American Cancer Society, seminar on tumor vaccines, Rye, New York November 19,20,21, 1959.

Perspectives in Virology II Symposium, New York City, New York, January 25, 26, 1960.

Symposium on Phenomena of the Tumor Viruses, New York City, New York, March 25, 26, 1960.

Serial No. NIAID - 68-A

1. Infectious Diseases
2. Virus & Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Sero-epidemiology of Virus Infections

Principal Investigator: Dr. R. J. Huebner

Other Investigators: Dr. W. P. Rowe, Dr. L. Rosen, Dr. R. M. Chanock, Dr. F. R. Abinanti, and Mr. H. C. Turner

Cooperating Units: NINDB, Collaborative study on cerebral palsy, mental retardation, and other neurological and sensory disorders of infancy and childhood (Dr. Richard Masland).

Man Years (calendar year 1959):

Total:	52/12
Professional:	4/12
Other:	48/12

Project Description:

Objectives: To apply newly developed viral serologic methods to a broad and integrated study of the roles of numerous newly recognized viruses in the high morbidity of acute undifferentiated respiratory diseases, chronic diseases, birth defects, and cancer. To enlarge in every way possible, by technical development and field trials, the usefulness of serologic methods for studying the natural histories and disease repertory of viral infections.

Methods Employed: Simplified serologic techniques, including complement fixation tests, hemagglutination-inhibition, hemadsorption, neutralization, and tissue culture neutralization tests are now available for the diagnosis of nearly 100 common viral diseases.

Part B Included: Yes [X] No [ ]

The first major problem has been the technical one of the commercial production of standardized and otherwise suitable antigens and virus suspensions and antiserum for such tests. The next problem is largely a logistic one which can be solved only by well supervised large-scale performance of tests; this means welding new and inexperienced workers in reliable and productive new virus serology teams -- comparable to the one already established in LID.

Human serums for viral diagnosis and retrospective analysis of viral experiences have been and will be available from our current studies of ill children and adults having common acute undifferentiated respiratory diseases; from epidemiological studies of special virus disease problems of contemporary interest to LID. Serial bleedings are being taken from mothers during the course of pregnancy and from the infants at and after birth, as outlined in the Collaborative Study on cerebral palsy, mental retardation, and other neurological and sensory disorders of infancy and childhood, of NINDB. Many of these latter sera should be available for testing during the next calendar year.

Major Findings: Viral complement-fixing antigens have been prepared for more than 40 viruses by Microbiological Associates, Inc., in consultation with the virologists in the Virus Section, LID.* Antigens have been titered in both laboratories and the acceptable ones have been produced in large quantities, i.e., from 100 to 2000 ml. amounts. These include complement-fixing antigens for: adenovirus (common antigen), herpes simplex, respiratory syncytial, influenza A, B, C, mumps, parainfluenza (3 types), poliovirus (3 types), ECHO viruses (22 types), Coxsackie A and B viruses (at least 8 types), and measles. Similarly, antigens for use in HI tests have also been produced for 17 adenoviruses, reoviruses, and the various myxoviruses. Twelve antigens capable of detecting antibody responses to 27 adenoviruses, 3 influenza viruses, 3 parainfluenza viruses, respiratory syncytial virus, reoviruses, mumps, salivary gland virus, Q fever, psittacosis, and others have already been incorporated in our routine tests of paired sera from patients with respiratory disease observed in three study populations - namely, Children's Hospital, Junior Village, and Parris Island. (See R. M. Chanock's project reports Nos. 69 and 69-A).

Two local Q fever cases were identified amongst Children's Hospital respiratory disease cases. Several outbreaks of respiratory syncytial virus were characterized exclusively by serology, as was an outbreak of mumps with unusual clinical aspects. Other outbreaks of myxoviruses, particularly the new parainfluenza M-25 (see Johnson's project report No. 73) were also characterized in large part by serology.

The prevalences of parainfluenza 3 infections in cattle of all ages, and from various geographic areas were established by serology. Similar information is now being acquired for bovine adenoviruses and reoviruses.

Recently the discovery of numerous extrinsic contaminating viruses in mouse cancer passage materials, and numerous latent viruses in mouse breeding colonies, led to the development of CF and HI tests for recognizing some of them (see Hartley's reports Nos. 71 and 72). Comparatively simple CF and HI tests permit large scale serological sampling of mouse breeding colonies for the presence of those extrinsic viruses which most commonly contaminate tumor and tumor-virus study systems. Such information promises to be invaluable in the eventual development of specific virus-free mouse colonies, and for recognition of contaminating, possibly interfering, viruses in tumor virus passage materials.

Significance to Program of the Institute: Serologic epidemiology of virus diseases represents the only method by which the importance of some viruses in human disease can be determined. This is particularly true of viruses that are difficult to isolate or viruses whose pathogenic activity may be difficult to determine, or whose effects may be delayed or not recognized until long after infection, as is the case of birth defects and animal cancers. Opportunities for extensive sero-epidemiology with a broad spectrum of viral antigens should soon provide exceptional opportunities for determining comparative prevalences of many viruses in many different epidemiological groups; thus the effects of age, sex, socio-economic, geographic, and seasonal factors can be evaluated.

Collaboration with NINDB in this study will enable us to determine the value of serologic epidemiology in defining the clinical importance of 70 to 80 new viruses of man, something which was impossible to conceive of only several years ago, because of the lack of tools available at that time.

Proposed Course of the Project: During the calendar year 1960 virus serology will gradually be expanded and broadened. The space available (now exceedingly limited) we hope will be increased, tests and reagents will be fully evaluated and preliminary definitive tests of serial specimens from NINDB will begin -- as dictated by the availability of information on birth defects. Since the chief function of this largely technological group is to carry out sero-diagnostic tests, it would be a logical development for this group to gradually take on an additional closely related service and function, namely, that of identification and typing of viruses which conceivably could be expanded with additional space and proper personnel to provide services for the entire NIH intramural program.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this Project:

Huebner, R.J.: 70 newly recognized viruses in man. Public Health Reports, 74: 6-12, 1959.

Huebner, R.J.: Coxsackie and ECHO viral infections. Cecil & Loeb: Textbook of Medicine, 1959.

Hoerlein, A.B., Mansfield, M E., Abinanti, F.R., and Huebner, R.J.: Studies of shipping fever of cattle. I. Para-influenza 3 virus antibodies in feeder calves. J. of Amer. Vet. Med. Assn., 135: 153-160, 1959.

Huebner, R.J.: Virus infection and virus disease: General considerations. The Merck Manual. In press.

Halonen, P., Rosen, L., and Huebner, R.J.: Homologous and Heterologous complement fixing antibody in persons infected with ECHO Coxsackie and poliomyelitis viruses. Proc. Soc. Exper. Biol. & Med., 101: 236-241, 1959.

Honors and Awards Relating to this Project:

Appointed to Committee on Enteroviruses, the National Foundation.

Appointed to Organizing Committee on "Perspectives in Virology".

Appointed to Adenovirus Committee, V&R Study Section, NIH.

Appointed to Technical Committee on Acute Respiratory Diseases, American Trudeau Society of the American Tuberculosis Association.

Appointed to National Research Council Committee Advisory to the U.S Army Chemical Corps.

Appointed consultant to the Clinique de Puericulture de la Faculte de Medecine de Paris, and to the Hopital Saint-Vincent-De-Paul.

Elected to the Societe Francaise de Microbiologie.

National Academy of Sciences, AEC seminar on "Low Level Effects of Radiation".

Epidemiology seminar for Veterinarians, Atlanta, Georgia, "Virus Induced Tumors".

Medical and Surgical Symposium, Watts Hospital, Durham, North Carolina

Ross Symposium - Perspectives in Pediatric Virology, Children's Hospital, Washington, D.C.

Annual Association Meeting of Radiologists, Washington, D.C., "Viruses and Cancer".

Annual Meeting of the Society of Experimental Biology and Medicine, Bethesda, Md., - dinner speaker.

Lecture on Virus Diseases, USPHS Lecture Series, Staten Island USPHS Hospital.

International Congress of Pediatrics, Montreal - speaker and panel moderator.

American Cancer Society Meeting, Rye, New York - seminar on cancer viruses.

Lobund Institute, U. of Notre Dame, Indiana - seminar on cancer viruses.

Seminar on "Respiratory Virus Diseases in Livestock", Pfizer, Terra Haute, Indiana.

Awards.

Alumni Merit Award, St. Louis University.

Serial No. NIAID - 69
1. Infectious Diseases
2. Virus and Rickettsial Dis.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Etiology of Primary Atypical Pneumonia: The Biology and Natural History of the Agent (Eaton) Associated with Cold Agglutinin Positive Atypical Pneumonia

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Dr. Maryjane K. Cook, Dr. Robert J. Huebner

Cooperating Units: Dr. J. Kingston, Dr. Henry Bloom, Dr. M.A. Mufson, and Dr. F. Gordon, Bureau of Medicine and Naval Medical Research Institute, U.S. Navy

Man Years (calendar year 1959):

Total:	50/12
Professional:	10/12
Other:	40/12

Project Description:

Objectives: 1) To characterize the agent first described by Eaton as to its capacity for growth in various host systems and to quantitate such growth when it occurs. 2) To employ the information gained in objective 1 to develop serologic tools other than fluorescent antibody microscopy for use in serodiagnoses and epidemiologic surveys. 3) To study the natural history of infection with Eaton agent in children and among military recruits, and continue the search for other viral agents causing pneumonia.

Major Findings: A) Study of infection by Eaton agent has been hampered by the difficult and time-consuming techniques required at present for the determination and quantitation of antibody and for isolation of the agent from infected individuals. For antibody studies infected chick embryos must be sectioned in a cryostat (microtome housed in a deep freeze). These sections are then used for the detection of antibody employing the fluorescent antibody technique. Until the present no experimental host other than the 13 day chick embryo has been available for serial passage of this agent.

Part B Included Yes [X] No [ ]

Recently, working with Dr. Gordon's group at the Naval Medical Research Institute, we have been able to grow Eaton agent in tissue cultures derived from a 4-day old chick embryo yolk sac endodermal cells. The agent has been passaged 5 times in these cells and tissue culture fluids have been shown to contain 100 infectious doses per ml. by back titration in eggs. Studies of the optimum conditions for growth and the quantitative aspects of multiplication are now in progress. Thus far attempts to detect a complement-fixing antigen have been unsuccessful.

B) The role of Eaton agent in human respiratory disease has been a controversial one since its first recognition in 1943. Therefore, studies employing the fluorescent antibody technique as an epidemiologic tool were undertaken in this laboratory in an attempt to clarify the status of this agent. Studies in adults indicated that approximately 85% of cold agglutinin and/or streptococcus MG positive sporadic atypical pneumonia and 25% of cold agglutinin, streptococcus MG negative pneumonia were associated with Eaton infection as determined by fluorescent antibody. The illnesses in these patients covered a 13 year period and were from diverse geographic localities both in and outside the United States. Eaton agent was recovered from 2 of 10 individuals in this group from whom frozen specimens were available.

A study of children hospitalized with lower respiratory tract disease over a 16-month period indicated that 10% of such illness was associated with infection with Eaton agent. Analysis of the serologic findings in children with etiologically undiagnosed illness, in children with respiratory illness associated with other viruses (para-influenza, influenza, adenovirus, etc.), and in control children without respiratory disease suggested that Eaton agent played an etiologic role in a significant proportion of illness in the first group.

Studies carried out at a Marine recruit training center indicated that approximately 70% of all clinically diagnosed pneumonia was associated with Eaton infection. In addition, 23% of the febrile respiratory illness without pneumonia was associated with this agent. Comparable controls (recruits free of respiratory illness) had a significantly lower rate of infection (6%). During the 12-week training period one recruit in every 100 was hospitalized with an Eaton positive pneumonia. Infection with Eaton agent occurred in 12 of every 100 recruits during the training cycle. These findings indicate that one clinically recognizable pneumonia occurs for every 12 infections, a high rate of pneumonitis when compared with other respiratory agents. Important information bearing on the spread and communicability of Eaton infection was gained during this study. Adenovirus infection occurred at a very low rate during this period and thus did not complicate the picture of Eaton infection. Other known respiratory viruses were also inactive.

Significance to the Program of the Institute: As demonstrated during the last year the consequences of infection with Eaton agent appear to be of considerable public health importance in the pediatric age group as well as in military recruits, the only groups so far studied. The importance of this agent in other populations is a pressing problem and germane to the program of our Institute.

Proposed Course of Project: One of the unsolved problems of Eaton infection is the effect of chemotherapy upon the course of illness. A double-blind controlled chemotherapy study to test the efficacy of Declomycin is now in progress at the Parris Island Marine Recruit Center in collaboration with the Bureau of Medicine of the United States Navy. The high rate of Eaton positive pneumonia at this base provides an unparalleled opportunity to carry out such a study.

Study of the agent in tissue culture and attempts to develop simpler serologic test procedures will continue. If sufficient quantities of virus can be obtained in tissue culture an attempt will be made to prepare an inactivated vaccine which can then be tested at Parris Island Marine Recruit Center.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R.M., Cook, M.K., Fox, H.H., Parrott, R.H., and Huebner, R.J.: Serologic Evidence of infection with Eaton agent in childhood lower respiratory illness. N.E.J.M. In press.

Johnson, R.T., Cook, M.K., Chanock, R.M., Buescher, E.L.: Family outbreak of primary atypical pneumonia associated with the Eaton agent. N.E.J.M. In press.

Cook, M.K., Chanock, R.M., Fox, H.H., Buescher, E.L., Johnson, R.T., and Huebner, R.J.: Studies on the role of Eaton agent in lower respiratory tract illness. Evidence for infection in adults. British Med. J. In press.

Serial No. [illegible]
1. Infectious Diseases
2. Virus & Rickettsial [illegible]
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Viruses as Causes of Respiratory Illness in Infancy and Early Childhood.

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Mrs. Karl M. Johnson, Maryjane K. Cook, Robert J. Huebner, and Albert Z. Kapikian

Cooperating Units: Dr. Robert Parrott, Children's Hospital Research Foundation, Washington, D. C.

Man Years (calendar year 1959):
Total: 30/12
Professional : 5/12
Other: 25/12

Project Description:

Objectives: 1. To search for new agents responsible for respiratory illness in infancy and childhood. 2. To delineate the epidemiology of viruses which have recently been associated with respiratory illness. 3. To continue the study of certain well established respiratory viruses, i.e., their epidemiology and contribution to the overall respiratory disease experience during infancy and childhood.

Methods Employed: Infants and children with respiratory illness and suitable controls without such illness will be studied at Children's Hospital of D. C. The severe lower respiratory syndromes will be investigated in hospitalized patients while the milder febrile respiratory disease syndromes will be studied among clinic patients.

Part B included: Yes [X] No [ ]

The main emphasis will be on the use of various tissue culture systems and the fluorescent antibody technique for virus isolation. An attempt will be made to inoculate specimens from patients directly into tissue culture without prior freezing and thawing since such treatment appears to rapidly inactivate respiratory syncytial virus and the current strains of influenza B. Fluorescent antibody techniques will be applied to the search for agents which may possibly grow in tissue culture without causing cell destructive effects.

Major Findings:

Para-influenza viruses: During the past year the para-influenza viruses types 1 and 3, which were first isolated in this laboratory, have continued to be a significant cause of respiratory illness in children. Over a two-year period in all seasons these agents have been recovered from 6% of children with all types of respiratory illness and serologic studies have indicated their contribution to illness may be as high as 26% for severe hospitalizable respiratory disease. These viruses are associated with 40% of acute laryngotracheobronchitis (croup) which is the most serious type of respiratory illness occurring in infancy and early childhood. The influenza and adenoviruses contribute significantly to the overall respiratory picture, but at a lower rate than the para-influenza viruses. Employing serologic techniques all these viruses (para-influenza, influenza, and adenoviruses) account for approximately 36% of all hospitalizable respiratory illness in childhood. Recent studies have indicated that the newly recognized respiratory syncytial virus (CCA) may be an important agent in childhood illness. Serologic studies carried out from November 1958 through April 1959 on 445 children with hospitalizable respiratory illness revealed that infection with respiratory syncytial virus had occurred in 19% of the group with a peak incidence of 32% during January 1959.

Other viruses: Adenoviruses, enteroviruses and PAP virus have also been demonstrated during these studies. When all of these and other experiences can be fully elucidated -- the question of the total amount of respiratory illness explainable by known (and, of course, demonstrable) viruses can be examined.

Significance to the Program of the Institute: Respiratory disease is the most common infectious ailment of man. Such infections occur most commonly and in their severest form during infancy and early childhood. Detailed knowledge of the agents responsible for respiratory disease and their natural history are a necessary prelude to attempts at either immunoprophylaxis or chemotherapy. As has been shown with the para-influenza viruses the benefits to be derived from studies among children, are not limited to this age group since the newer respiratory agents discovered in childhood illness are also associated with infections which may produce a generally milder illness in adults.

Proposed Course of the Project: It is planned that this study will continue for a period of several years, since a large segment of childhood respiratory illness remains to be elucidated. In addition, viruses responsible for respiratory illness vary at different times and in different localities. Therefore, surveillance should continue in order to comprehend a larger picture.

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Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Chanock, R.M., Vargosko, A., Luckey, A., Cook, M.K., Kapikian, A.Z. and Reichelderfer, T. Association of hemadsorption viruses with respiratory illness in childhood. J.A.M.A., 169: 548-554, 1959.

Parrott, R.H., Vargosko, A., Luckey, A., Kim, H.W., Cumming, C., and Chanock, R.M. Clinical features of infection with hemadsorption viruses. New England J. Med., 260: 731-738, 1959.

Vargosko, A.J., Chanock, R.M., Huebner, R.J., Luckey, A.H., Kim, H.W., Cumming, C., and Parrott, R.H. Association of type 2 hemadsorption (parainfluenza 1) virus and Asian influenza A virus with infection in croup. New England J. Med., 261: 1-9, 1959.

Honors and Awards Relating to this Project:

Elected Associate Member Acute Respiratory Disease Commission, Armed Forces Epidemiology Board.

Proj No. NIAID-66
1. Infectious Diseases
2. Virus & Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of respiratory virus illness in a welfare orphanage.

Principal Investigator: Dr. Robert M. Chanock

Other Investigators: Dr. K. M. Johnson, Dr. A.Z. Kapikian, Dr. R. I. Huebner

Cooperating Units: Dr. J.A. Bell, Epidemiology Section, LID
Dr. F.M. Mastrota, " " " and
D.C. Welfare Department

Man Years (calendar year 1959):

Total:	62/12
Professional:	12/12
Other:	50/12

Project Description:

Objectives: To elucidate the natural history of certain newly discovered respiratory viruses by means of a longitudinal study of an orphanage population.

Methods Employed: The population under study is an orphanage nursery in the District of Columbia (see Project NIAID-66) whose average population is 90-100 children between the ages of 6 months and 3 years. The turnover of infants and children is rather rapid and, as a result, a great deal of respiratory illness occurs in this population throughout the year.

Rectal temperatures are taken twice a day and the population is carefully observed by a full-time pediatrician who also provides medical care. Throat and rectal swabs are collected three times a week. Serum specimens are obtained on admission and discharge and at various intervals between. Throat swabs are tested in monkey kidney tissue culture for the presence of various myxoviruses. Serum specimens are tested for antibody to the various myxoviruses and other respiratory agents by the neutralization, hemagglutination-inhibition, or complement fixation technique. Laboratory data are then correlated with clinical,

Part B included Yes [X] No [ ]

bacteriological, and epidemiological information in an effort to determine qualitative and quantitative virus-disease relationships.

Major Findings:

Severity of Primary Infections with Parainfluenza 3: Three outbreaks of parainfluenza 3 virus infection were observed during the past year. The outbreaks occurred in December, 1958, January, 1959; March, 1959; and May-August, 1959. During these outbreaks, parainfluenza 3 virus was recovered from 123 infants and children. Approximately 10% of all infected children developed a clinically recognizable pneumonia. The occurrence of pneumonia associated with a parainfluenza 3 infection was limited almost exclusively to those infants and children who were undergoing their first experience with this agent and thus, for this group, the rate was 20%. Infection was associated with a febrile illness in approximately 80% of children who were without neutralizing antibody prior to exposure. Febrile illness occurred, but significantly less often among children who possessed neutralizing antibody prior to infection. These findings provide excellent support for the contention that parainfluenza 3 virus causes febrile respiratory illness.

Reinfection with Parainfluenza 3: The frequent observation of infection in individuals already possessing neutralizing antibody suggested that reinfection with this agent is not an uncommon event. The occurrence of such reinfection within a 1 to 6 month period was documented for 16 children from whom virus was recovered during the first or second outbreak and from whom virus was again recovered during the second or third outbreak. Serological studies confirmed the occurrence of both infections in these individuals and antigenic analysis failed to indicate any differences in the viruses recovered during the first or second infection. Febrile illness was generally associated with the first infection, while the second infection usually occurred without concurrent illness. The risk of reinfection for children in residence during a subsequent outbreak was approximately 20%. The ease with which reinfection occurs with this virus suggests that reinfection after a long interval, when neutralizing antibody may have decreased to very low levels, may be associated with mild upper respiratory illness of the type described as the common cold. Support for this hypothesis is the finding that adult volunteers with neutralizing antibody respond to infection with a "common cold"-like picture when virus is given (Kapikian, Chanock, Huebner, Ward (unpublished), and D.A.J. Tyrrell et al., Brit. Med. J., Nov. 7, 1959).

Respiratory Syncytial Virus: A rather abrupt outbreak of febrile illness in which 39 of the 69 children in residence had onsets of fever within a 4-day period was apparently associated with respiratory syncytial virus. Serological tests indicated that approximately 90% of children in residence during that interval developed antibody for respiratory syncytial virus. Unfortunately, virus was recovered from only one of the children. The great difficulty in recovering this agent reported previously makes any definitive epidemiologic association of infection with illness difficult. Until better methods for virus recovery are available, the evidence that this agent caused respiratory illness will likely remain tentative.

Significance to the Program of the Institute: The information gained during the parainfluenza 3 virus outbreaks has provided definitive evidence that this agent causes febrile respiratory illness and pneumonia. In addition, the quantitative aspects of infection in terms of how often febrile illness occurs during primary infection or reinfection, were elucidated for the first time. The occurrence and clinical consequences of reinfection were described for the first time. All of these findings are essential to our understanding of the natural history of the parainfluenza viruses and have a bearing on the practicality of various immunoprophylactic measures that will be attempted in the next few years. Implications of these findings to the problem of mild upper respiratory illness (common cold) in adults are also of some importance.

Proposed Course of the Project: The project will continue in an effort to gain a better understanding of the newer respiratory viruses. Information bearing on the natural history of the other parainfluenza viruses (types 1 and 2) and M-25 virus (which will probably be classified as parainfluenza 4) is sorely needed and can only be obtained by the type of longitudinal study possible at Jr. Village. It is hoped that opportunities to study the natural history of respiratory syncytial virus will also occur. Experimental vaccines for the parainfluenza viruses will be evaluated for their effectiveness in preventing infection and illness.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Honors and Awards relating to this project:

Invited to speak before Laboratory Section, American Public Health Association, on "Serologic Diagnosis of Parainfluenza Infection", based on outbreaks studied at Junior Village - October, 1959.

Serial No. NIAID-70
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Laboratory Studies of Newly Recognized Viruses Associated with Respiratory Illness

Principal Investigator: Dr. Maryjane K. Cook

Other Investigators: Dr. Robert M. Chanock, Dr. Robert J. Huebner, Dr. Karl M. Johnson, and Dr. Francis R. Abinanti

Cooperating Units: None

Man Years (calendar year 1959):
Total: 20/12
Professional: 10/12
Other: 10/12

Project Description:

Objectives: 1) To investigate the biologic and antigenic properties of the newly recognized para-influenza viruses associated with respiratory illness. 2) To determine the significance of shared antigens among the para-influenza viruses. 3) To determine if previous infection with heterologous para-influenza viruses affect the course of infection and virus multiplication.

Methods Employed: The standard serological tools of virology - neutralization, complement fixation, and hemagglutination inhibition - are employed to determine the antigenic specificity and existence of shared antigens among the para-influenza viruses. Hamsters are infected with various para-influenza viruses and the course of infection and lesion production quantitated. These animals are then challenged at 1 to 3 month intervals with the homologous or a heterologous para-influenza virus and the effect of previous infection upon virus multiplication is determined.

Part B Included Yes [X] No [ ]

Major Findings: The newer myxoviruses (hemadsorption type 1, hemadsorption type 2, and CA) were found to be unrelated antigenically to the influenza viruses types A, B, or C. In addition, the newer myxoviruses were shown to be distinct from each other in CF tests employing soluble, tissue culture, or egg fluid antigens. Although these agents were distinct, they appear to comprise a group with mumps and Newcastle disease virus in which common antigens exist, each member being related to at least one member and frequently to two or more members of the group. The occurrence of the common antigens plus certain biologic properties, such as the occurrence of hemolytic activity against certain types of erythrocytes, clearly sets these agents apart from the influenza viruses. The newer agents have been classified as para-influenza by an International Committee of Virus Nomenclature (see reference 2) - para-influenza 1 = hemadsorption type 2, para-influenza 2 = CA, and para-influenza 3 = hemadsorption type 1.

Antigenic sharing is not manifest in tests with serum from individuals or animals undergoing primary infection. However, if previous para-influenza antigenic experience has occurred, heterologous responses are more likely to occur following infection or immunization.

Antigenic distinctness of para-influenza 1 and 3 viruses was confirmed in hamster challenge experiments in which previous infection with one of these viruses did not modify subsequent response to the heterologous agent when it was administered 1 month later. Homologous protection was demonstrable for at least 3 months for para-influenza 3 virus, but after 1 month such protection was minimal for para-influenza 1 (HA-2) virus.

A study of para-influenza 3 viruses recovered from humans infected in various countries (U.S., Canada, Great Britain, France and Australia), and during different years (1955-1959) failed to indicate the existence of antigenic variation. This finding is of some importance when coupled with the demonstration that this agent is capable of readily infecting humans who possess antibody from previous infection. Thus, there appears to be little selective pressure exerted on this virus to mutate and change its antigenic configuration, an occurrence which is characteristic of influenza A viruses.

Significance of Program for the Institute: Knowledge of the antigenic properties of the para-influenza viruses is required in order to perform meaningful epidemiologic studies with these agents. The problem of antigenic overlap is important when attempts are made to interpret the results of serologic studies designed to measure the contribution of each agent to respiratory disease. The findings relevent to the broadening of serologic response in which antibody develops for viruses which the host has not experienced has implications both in the study of antigenic interrelationships and in the interpretation of the results of serologic tests employed for virus diagnoses and epidemiologic survey.

The finding of lack of resistance to homologous challenge with para-influenza 1 virus 1 month after primary infection has implications in both the natural history of this virus and in studies designed to test various methods of immunoprophylaxis.

Proposed Course of the Project: The possibility of antigenic variation in para-influenza 3 and also in para-influenza 1 and 2 viruses must continue to be investigated. The most pressing problem at the moment appears to be that of understanding the effect of previous infection and neutralizing antibody upon subsequent infection and the clinical consequences of such infection. Experimental animal host systems and quantitative descriptions of such infection in such hosts are now available to pursue this problem. Attempts will be made to obtain attenuated variants of the para-influenza viruses by growth on tissue cultures held at low temperatures of incubation (a procedure which has recently been successful with poliovirus). Such variants will be tested for their capacity to immunize and modify the consequences of subsequent infection.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Cook, M.K., Andrews, B.E., Fox, H.H., Turner, H.C., James, W.D., and Chanock, R.M.: Antigenic relationships among the "newer" myxoviruses (parainfluenza). Amer. J. Hyg., 69: 250-264, May, 1959.

Andrewes, C.H., Bang, F.B., Chanock, R.M., and Zhdanov, V.M.: Parainfluenza viruses 1, 2, and 3: Suggested names for recently described myxoviruses. Virology, 8: 130, May, 1959.

Honors and Awards Relating to this Project: NONE

Serial No. NIAID - 71
1. Infectious Diseases
2. Virus and Rickettsial Disease
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of Tumor-Producing Viruses

Principal Investigator(s): Dr. Wallace P. Rowe and Dr. Robert J. Huebner

Other Investigators: Dr. Janet W. Hartley, Dr. Isadore Brodsky, Dr. David O. White, Dr. Alexander Kohn

Cooperating Units: Dr. Herbert Kahler, and Dr. Lloyd W. Law, NCI

Man Years (calendar year 1959):
Total: 130/12
Professional: 30/12
Other: 100/12

Project Description:

Objectives: To characterize biologically, serologically, biophysically, and epidemiologically, viruses which produce tumors in animals.

Methods Employed: Application of standard virologic procedures for quantitation and characterization of animal tumor viruses, with particular emphasis on development of tissue culture and serologic procedures.

Major Findings:

A. Mouse polyoma virus. Work with this agent has been concerned with additional characterization of the agent, studies of the epidemiology of infection, and studies of the growth of the virus in different host systems.

(1) In newborn mice polyoma virus grows to high titer in many organs, particularly kidneys and salivary glands. Virus remains present throughout life, and is regularly present in tumors. The most sensitive method for recovery of virus from tumors is cultivation of the tissue in vitro.

Part B included: Yes ☒ No ☐

(2) In weanling mice, which are refractory to tumor development, the virus multiplies, but to lower titer than in newborns. Excretion of virus is minimal.

(3) In suckling hamsters, multiplication of virus is also much lower than in newborn mice, but tumor development is rapid. The virus disappears rapidly, and most tumors do not contain detectable virus.

(4) Extensive epidemiologic studies have disclosed that spontaneous polyoma virus infection of wild house mice is present in certain localized areas in New York City. Virus was recovered from wild mice and from floor sweepings containing nesting materials. No evidence of infection in other wild rodents was found.

(5) Variants of polyoma virus, differing in oncogenic potentiality and sensitivity to inhibitors of hemagglutination have been encountered and are under study.

(6) Studies of the relation of polyoma infection to occurrence of spontaneous tumors in infected laboratory mouse colonies have indicated no significant influence of past infection on tumor incidence. However, this was not unexpected, since natural infection of laboratory animals occurs mostly after the infant period and such infections almost never result in tumor production.

B. Friend mouse leukemia virus. A complement fixation procedure has been developed which appears to be specific. However, the virus is a poor antigen, and CF antibodies do not develop without hyperimmunization; consequently, the test cannot now, at least, be applied to epidemiologic studies. Growth studies have been made, and neutralization tests are being developed.

C. Visceral lymphomatosis virus of chickens. The virus isolated from the RPL 12 transplantable visceral lymphomatosis virus by Sharpless and Burmester has been studied in this laboratory in tissue cultures of chick embryo liver. Also serological surveys of chicken flocks and virus distribution studies in artificially inoculated chicks have been done. Antibodies to this agent are extremely prevalent among commercial chickens. In all flocks tested, 80 to 90% of animals had neutralizing antibody for the agent. The virus infects the baby chicks readily by tracheal instillation and produces primarily an enteric infection with large amounts of virus excreted in the droppings. It is highly contagious. Serologic studies are in progress to determine whether this virus is actually the visceral lymphomatosis virus or, as seems likely, some extraneous agent.

D. Studies of papilloma viruses. The Shope rabbit papilloma virus has been studied for the possibility of adapting it to growth in tissue culture and for the possibility of activating papilloma virus from the apparently non-virus carrying virus-induced carcinoma of the VX2. However, results to date have been essentially negative. Comparable studies are also being done with human wart material. Warts removed from volunteers are passed to volunteer recipients as well as to a wide variety of tissue cultures. Early results indicate successful takes in volunteers, but again no evidence of destructive effects on any tissue culture.

Significance to the Program of the Institute: The demonstration by Stewart, Eddy, and our laboratory that polyoma virus can be handled by standard virologic techniques is of the utmost importance to the field of microbiology in general. A virtual revolution has occurred in many major virus laboratories as a result of this finding; intensive efforts are being made in many areas to apply standard virologic techniques to the problems of the viral etiology of cancer. Thus, the possibility that the general virologist can make significant contributions to the tumor problem now appears very real, and consequently this program would appear to be of the utmost importance to the Institute.

Proposed course of the Project: Epidemiologic studies of polyoma virus and visceral lymphomatosis virus will be continued, with particular reference to reservoirs of infection and influence of the mode of acquisition of infection on the occurrence of tumors. Studies will continue to attempt to develop *in vitro* systems for recognition of other tumor viruses, particularly the Friend, Gross, and Schwartz leukemia agents, and the human, bovine, and rabbit papilloma viruses.

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Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Kahler, H., Rowe, W.P., Lloyd, B.J., and Hartley, J.W.: Electron microscopy of mouse parotid tumor (polyoma) virus. J. Nat. Cancer Inst. 1959, 22: 647-657.

Hartley, J.W., and Rowe, W.P: "Unmasking" of mouse polyoma virus hemagglutinin by heat and RDE. Virology, 1959, 7:249-250.

Rowe, W.P., Hartley, J.W., Estes, J.D., and Huebner, R.J.: Studies of mouse polyoma virus infection. I. Procedures for quantitation and detection of virus. J. Exp. Med., 1959, 109: 379-391.

Brodsky, I., Rowe, W.P., Hartley, J.W., and Lane, W.T.: Studies of mouse polyoma virus infection. II. Virus stability. J. Exp. Med., 1959, 109: 439-447.

Rowe, W.P., Hartley, J.W., Law, L.W., and Huebner, R.J.: Studies of mouse polyoma virus infection. III. Distribution of antibodies in laboratory mouse colonies. J. Exp. Med., 1959, 109: 449-462.

Hartley, J.W., Rowe, W.P., Chanock, R.M., and Andrews, B.E.: Studies of mouse polyoma virus infection. IV. Evidence for mucoprotein erythrocyte receptors in polyoma virus hemagglutination. J. Exp. Med., 1959, 110: 81-91.

Levy, H.B., and Brodsky, I.: The effect of a leukemia virus on phosphorous uptake by mouse spleen. Cancer Research., 1959, 19: 477-78.

Rowe, W.P., and Brodsky, I.: A graded-response assay for the Friend leukemia virus. J. Nat. Cancer Inst., in press.

Law, L.W., Dawe, C.J., Rowe, W.P., Hartley, J.W.: Antibody status of mice in response to their litters to parotid tumor virus. Nature, 1959, 184: 420.

Honors and Awards Relating to this Project: NONE

Serial No. NIAID 71-A
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Continuing Studies of Adenoviruses and Salivary Gland Viruses as Examples of Latent Viruses.

Principal Investigator: Dr. Wallace P. Rowe

Other Investigators: Dr. Janet W. Hartley and Dr. Robert J. Huebner

Cooperating Units: None

Man Years (calendar year 1959)
Total: 2/12
Professional: 1/12
Other: 1/12

Project Description:

Objectives: To characterize the salivary gland viruses and adenoviruses, and to determine their epidemiology and clinical importance.

Methods Employed: Serologic epidemiology and virus isolation in tissue culture. Studies of the biology of adenoviruses and salivary gland viruses of lower animals.

Major Findings: Salivary gland virus was isolated from two cases of neonatal cytomegalic inclusion disease. Serologic studies on a case of fatal disseminated SGV infection in a leukemic adult indicated that the infection was primary, and no activation of a latent infection.

Mouse salivary gland virus infection has been found to be highly prevalent in wild mice, virus being found in 60-80% of Mus musculus in both rural and urban areas.

Part B included Yes ☐ No ☒

We have assisted Mr. Kasel, of the Laboratory of Clinical Investigations, in elucidating an adenovirus enzyme affecting erythrocyte receptors.

Significance to the Program of the Institute: Salivary gland virus infection is of much interest as a clinical problem in pediatrics, and as an exceptional model of latent viral infection.

Proposed Course of the Project: Serologic epidemiologic studies of human SGV infection will be continued, particularly in relation to congenital anomalies and neonatal disease. The high prevalence (60-80%) of infection in wild mice provides an excellent opportunity for defining the natural history of this virus in natural mouse populations, in the same way (and at the same time) as the field studies on polyoma (see projects Nos. 68 and 71).

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of Mouse Viruses

Principal Investigator: Dr. Janet W. Hartley, Dr. Wallace P. Rowe

Other Investigators: Mr. Lee Smith, Dr. Robert J. Huebner

Cooperating Units: None

Man Years (calendar year 1959)
Total: 18/12
Professional: 6/12
Other: 12/12

Project Description:

Objectives: To characterize and develop methods for recognizing infection with known and newly recognized mouse viruses for the purpose of defining and eliminating the extrinsic murine agents which contaminate and complicate studies of mouse tumor viruses.

Methods Employed: Serologic, tissue culture, and animal pathogenicity studies. Attempts to isolate agents from laboratory and wild mice, and from spontaneous, induced, and transplanted mouse tumors. Studies of the epidemiology of the agents under various natural and artificial conditions.

Major Findings:

1. Of the known mouse viruses, reagents for serological tests have been prepared for polyoma, mouse salivary gland, K, PVM, and LCM viruses; such materials for the reputed strains of mouse hepatitis virus are in preparation. In addition, during studies employing laboratory and wild mice several isolations have been made of apparently previously undescribed agents. Two of these have been sufficiently well characterized to warrant a brief description:

Part B included: Yes [ ] No [X]

a. From General Purpose Swiss mice in the NIH colony a virus has been recovered which produces a fatal infection in newborn to 10-day old mice; histological lesions include inflammatory responses in heart, adrenal, kidney, and brown fat, accompanied by intranuclear inclusion bodies. The agent may be grown in mouse embryo tissue culture in which it produces cytopathic changes and inclusions; it is ether resistant, approximately 100 mmu in diameter, and produces a soluble complement fixing antigen. The infection in weanling mice is highly contagious, as evidenced by development of antibody in uninoculated contacts; virus has been recovered from urine and nasal mucosa of experimentally infected weanlings but not from saliva or feces. This agent has been provisionally designated as myocarditis-adrenalitis-steatitis (MAS) virus.

b. Three isolations of a virus related to type 2 reovirus have been made from wild mice in New York City. The agent produces cytopathic changes in mouse embryo and monkey kidney tissue culture, with characteristic intracytoplasmic inclusion bodies; infected tissue culture fluids contain group-reactive complement fixing antigen and hemagglutinin for human type O erythrocytes.

2. In preliminary surveys for complement fixing antibody to K and MAS viruses carried out for a number of strains of mice in several colonies, it appears that K virus infection is wide spread but of generally low incidence whereas MAS virus infection has been found prevalent only in the NIH colony General Purpose Swiss mice (approximately 50% of retired breeders).

3. Use of the mouse antibody production (MAP) test developed for polyoma virus is being evaluated for the several agents under study as an aid in rapid detection and identification of naturally occurring mouse viruses. It is highly satisfactory for the MAS virus and evidence to date indicates its usefulness for K, mouse reovirus and LCM.

Significance to the Program of the Institute: In the widening studies of spontaneous, induced or transplanted mouse tumors it is becoming increasingly necessary to define natural virus experience in mouse populations and to determine their role in tumor etiology. Such information can be acquired only through intensive application of virologic methods in virus isolation attempts followed, when successful, by preparation of specific reagents and development of serologic techniques for rapid identification of isolates. Serologic techniques, where effective, are invaluable in defining the experiences of experimental and wild mouse colonies. The definition and elimination of extrinsic viruses in tumor passage materials and in the breeding colonies furnishing the host systems as well represents the first objective that must be achieved before precise or even accurate information can be obtained about tumor viruses studied in such systems.

Proposed Course of the Project: Tissue culture, mouse pathogenicity, and mouse antibody production tests will be evaluated and used in virus isolation attempts from normal and tumor-bearing mice from a variety of sources. Serological surveys of laboratory and wild mouse populations will be continued and extended as additional reagents become available. Attempts will be made to determine the role of naturally occurring mouse viruses with respect to oncogenic capacity, either as primary or secondary incitors, or as possible interfering agents in tumor formation.

Serial No. NIAID - 75
1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Viral Respiratory Illness on a University Campus.

Principal Investigator: Dr. Karl M. Johnson

Other Investigators: Dr. Albert Z. Kapikian, Dr. Robert M. Chanock, and Dr. Robert J. Huebner

Cooperating Units: Dr. L. M. Dyke, Dr. R. E. Iler, University of Maryland, Student Health Service

Man Years (calendar year 1959)
Total: 30/12
Professional: 5/12
Other: 25/12

Project Description:

Objectives: To determine the importance of certain newer respiratory viruses in adult illness. Study specifically designed to compare adult experience with that seen in children in comparable geographic area and at similar time.

Methods Employed: Students reporting to University of Maryland Health Service on Tuesdays and Thursdays with complaints of respiratory nature are examined by two participants in study. Histories of illness are obtained, physical examinations performed and throat specimens collected. Acute and 3-week convalescent blood samples are drawn. Non-respiratory control patients are obtained where possible.

Throat swabs are tested in monkey kidney and Chang liver tissue cultures. Paired serum samples are used in viral antibody studies.

Part B included: Yes /X/ No /___/

Major Findings: 1. In general, the rates of virus isolation and demonstration of complement-fixing antibody responses to 11 different respiratory viruses were much lower than in young children studied during the same period of time. With two exceptions, a total of 50 illnesses in the 6-week period from November 3 to December 15, 1958, were not explainable by tests for known viruses.

However, following the Christmas recess, a sharp increase in patients seen at the Health Service was accompanied by the appearance of serologic response to three different viruses. Although virus isolation was not achieved (probably due to problems of collecting and holding specimens), rises in complement-fixing antibodies were demonstrated in January and February, 1959 against influenza, para-influenza 1, and respiratory syncytial virus. Although numbers of individuals were not comparable, the percentage of individuals reacting to influenza B and to respiratory syncytial agents were similar to those seen in children during this time.

Preliminary analysis reveals that it is unlikely that illnesses associated with evidence of a given virus infection can be linked with any particular set of clinical findings.

2. In November, 1958, a new hemadsorption virus was recovered from a student with an illness indistinguishable from a "common cold". This virus has been characterized and was shown also to have infected a group of children in an orphanage at about the same time. In all, 35 strains were recovered. The new virus, called M-25 virus, has been shown to be a myxovirus, 150-200 mu in size. So far, it has been grown only in tissue cultures of primary origin. Attempts at egg adaptation have been unsuccessful. Guinea pigs and hamsters may be infected but no illness is produced. Complement-fixing antigen has been prepared in monkey kidney tissue culture, but hemagglutinin has not been demonstrated in sufficient concentration for serologic utilization. This agent will be proposed as a prototype of para-influenza 4.

Significance to the Program of the Institute: This project has resulted in the discovery of yet another new myxovirus, possibly associated with a portion of the huge mass of respiratory viral infections. It has also indicated that careful study of respiratory illness in adults may reasonably be expected to yield evidence for association of certain of the newer viruses with such disease.

Proposed Course of the Project: Further laboratory and field observations on M-25 virus will be carried out. A volunteer experiment is contemplated. The study of viral respiratory disease in adults will be expanded, under conditions that will provide more precise and extensive data.

Studies of JH and 2060 viruses, suspended when M-25 virus was encountered, will be resumed at an appropriate time.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Johnson, K.M., Chanock, R.M., Cook, M.K., and Huebner, R.J. Studies of a new human hemadsorption virus I. Isolation, properties and characterization. Amer. J. of Hyg. (in press - January, 1960).

Honors and Awards relating to this project:

None

Serial No. NIAID - 74
1. Infectious Diseases
2. Virus & Rickettsial
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The role of viruses in respiratory diseases of domestic animals.

Principal Investigator: Dr. Francis R. Abinanti

Other Investigators: Dr. Robert J. Huebner;
Dr. Robert Byrne, Division of Veterinary Science, U. of Maryland;
Dr. A. B. Hoerlein, College of Veterinary Medicine, U. of Illinois.

Cooperating Units: Dairy Science Department, U. of Maryland

Man Years (calendar year 1959):

Total:	12/24
Professional:	6/12
Other:	6/12

Project Description:

Objectives: (1) The isolation of viral agents from the respiratory tract of normal cattle and cattle with respiratory disease; (2) to assess the role of myxovirus parainfluenza 3 virus in respiratory diseases of cattle; and, (3) to determine the relationships of the human and bovine strains of myxovirus parainfluenza 3.

Methods Employed: Specimens of nasal secretions were taken from cattle and inoculated onto tissue cultures of bovine and monkey kidney. The viruses isolated are characterized and compared to known viral agents of man, cattle, and other animals. Serial samples of blood are taken from normal cattle and acute and convalescent specimens from diseased cattle, and hemagglutination-inhibition, complement fixation, and neutralization tests are used to develop information concerning the occurrence, prevalence, and spread of viruses.

Part B included Yes [X] No [ ]

Major Findings: (1) Eighteen recoveries of myxovirus parainfluenza 3 virus have been made from cattle, most of which were suffering from respiratory disease. Evidence was found, serologically, of a relationship of this virus to the outbreaks of disease. Extensive serological studies produced evidence that infection with this virus is widespread. In a preliminary test, young cattle developed good levels of antibody to experimental vaccines prepared against both human and bovine strains of parainfluenza 3, and appeared to be refractory to an aerosol challenge by live virus.

(2) Several large groups of cattle were vaccinated under field conditions with commercially produced parainfluenza 3 (bovine strain) vaccines. These vaccines also produced good levels of circulating antibody. Unfortunately, in these field trials little or no respiratory disease has so far occurred. Insufficient funds prevented sufficiently extensive studies to insure a valid study.

(3) Comparative studies of the human and bovine strains of the virus showed that they can be distinguished by means of prototype guinea pig sera in the HI test.

(4) Infectious bovine rhinotracheitis, a virus usually recovered from cattle with respiratory disease, was recovered from cattle involved in a large outbreak of bovine conjunctivitis - attended by little or no respiratory disease. The virus strain recovered produced conjunctivitis and minimal respiratory illness in experimentally inoculated calves.

Significance to the Program of the Institute: The discovery of viruses in domestic animals which are similar or identical to those responsible for large amounts of human respiratory disease is of obvious importance. Furthermore, the study of respiratory disease in domestic animals and the efficacy of antivira vaccines in controlling such illnesses provide incomparable models of similar problems in man. In addition, solutions to some of the common viral diseases of food animals have great significance for human health in many areas of the world where animal protein is deficient. These domestic animal viruses suggest the existence of widespread unique and new zoonoses; because of the meagerness of available information, perhaps of more importance and thus deserving of more support than older, better understood zoonoses.

Proposed Course of Project: The work on this project at the Institute (which is critical to its continuation) has been done chiefly by principal investigator with the technical assistance of one trainee, employed by NINDB. Since laboratory work on the NINDB program must soon be activated, there is considerable likelihood that this new project will be terminated for lack of realistic support.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Abinanti, F.R., and Huebner, R.J.: The serological relationships of strains of parainfluenza 3 virus isolated from humans and cattle with respiratory disease. Virology, 8: 392-393, 1959.

Abinanti, F.R., Byrne, R.J., Watson, R.L., Poelma, L.J., Lucas, F.R., and Huebner, R.J.: Observations on infections of cattle with myxovirus para-influenza 3. Am. J. Hyg. (in press).

Hoerlein, A.B., Mansfield, M.E., Abinanti, F.R., and Huebner, R.J.: Studies of shipping fever of cattle. I. Para-influenza 3 virus antibodies in feeder calves. J. Amer. Vet. Med. Assn., 135: 153-160, 1959.

Honors and Awards relating to this project:

NONE

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-75
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

A. Project Title: Ecologic studies of fungi pathogenic for man.

Principal Investigator: C. W. Emmons

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1.2
Professional: 0.3
Other: 0.9

Project Description:

Objectives:

To find environmental habitats of fungi which cause systemic mycoses, to seek reasons for limitation of these fungi to their usual habitats, to estimate degree of resultant exposure of man to spores of these fungi and to apply information thus obtained to studies of the epidemiology of the systemic mycoses.

Methods Employed:

Samples of soil were collected from sites suspected of being sources of infection in individual cases of mycoses, from types of habitats known to harbor fungi in order to extend experience in this study and repeatedly in certain selected sites known to harbor pathogens in intensive studies of their ecology and associated saprophytic microflora. The specimens were processed by conventional methods of mouse inoculation with soil suspensions.

Major Findings:

Many additional environmental potential sources of infection for man have been found. The almost constant association of virulent strains of Cryptococcus neoformans and old deposits of pigeon manure (first observed by Emmons) has been reconfirmed in many

Part B included.

urban sites, including window ledges outside Federal office buildings. The presence throughout the year of Histoplasma (first shown by Emmons to be a saprophytic component of the microflora of soil and humus) has been verified in a site selected for intensive ecologic study. We have not been able to recognize a significant difference between the microflora of positive soil samples and those from areas a few feet distant, known by repeated sampling to be free of Histoplasma. However, we have isolated unusual and interesting fungi which are still under study.

Significance to bio-medical research and the program of the Institute:

The systemic mycoses are not contagious and each patient is infected by inhalation or mucosal or subcutaneous implantation of fungus spores from a saprophytic source in his environment. Additional information about the ecology of the fungi is needed for understanding of the epidemiology of the systemic mycoses and their control.

Proposed Course of Project:

This project will be continued indefinitely.

(Attachment I)

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Serial No. NIAID-75

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Emmons, C. W. Prevalence of Cryptococcus neoformans: relation to the pigeon (Columba livia). Public Health Reports. (In press).

Honors and Awards relating to this project:

None

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-76
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

D. Project Title: Identification and study of new and unusual fungi from mycoses.

Principal Investigator: C. W. Emmons

Other Investigators: William Jellison, Lie-Kian-Joe and many other collaborators who send fungi for identification.

Cooperating Units: Rocky Mountain Laboratory and many hospitals, diagnostic laboratories and individual physicians.

Man Years (calendar year 1959):

Total:	0.7
Professional:	0.3
Other:	0.4

Project Description:

Objectives:

Studies of new mycoses and their etiologic agents of unusual strains of pathogenic fungi and of the geographic distribution of mycoses. Support and encouragement of medical mycology in laboratories which lack trained mycologists.

Methods Employed:

Routine methods of fungus identification, adaptation of such methods when necessary, tests of pathogenicity and virulence in experimentally infected animals.

Part B included.

Major Findings:

Several unusual pathogens have been received and studied, the name "physomycosis" was proposed for the diseases caused by several species of the class Phycomycetes which are now more frequently recognized as causes of disease, but often lack specific and generic identification because diagnosed only after autopsy.

Significance to bio-medical research and the program of the Institute:

This combines a useful diagnostic service with an important research function because it brings under scrutiny unusual, interesting and important pathogens.

Proposed Course of Project:

This project will be continued indefinitely.

(Attachment I)

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Calendar Year 1959

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Lie-Kian-Joe, Njo-Injo Tjoei Eng, Sutoma Tjokronegoro, S. J. Schaafma, and C. W. Emmons. Phycomycosis of the Central Nervous System, associated with Diabetes Mellitus in Indonesia. A. J. Clin. Path. 32: 62-70, 1959.

Lie-Kian-Joe, Njo-Injo Tjoei Eng, Sutoma Tjokronegoro, and Chester W. Emmons. Phycomycosis (Mucormycosis) in Indonesia. Description of a case affecting the subcutaneous tissue. Am. J. Trop. Med. (In press).

Honors and Awards relating to this project:

None

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-76-a
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

B. Project Title: In vivo tests of new antimycotic drugs and antibiotics.

Principal Investigator: C. W. Emmons

Other Investigators: Prescott, Benjamin, Ph.D., Piggott, Willard, Utz, John P., M.D., and Andriole, Vincent, M. D. - LCI Serial #23-c and MPB Serial #64-a.

Cooperating Units: Laboratory of Clinical Investigations and Medical Physiological Bacteriology Section

Man Years (calendar year 1959):

Total:	1.2
Professional:	.2
Other:	1.0

Project Description:

Objectives:

To test the safety and efficacy of new drugs and antibiotics in treatment of experimentally infected animals.

Methods Employed:

Mice are infected intravenously with a dose of fungus cells determined in prior experimental work to be sufficient to kill untreated (control) mice in 14-21 days. Treated mice are given test drugs at doses up to tolerance and effectiveness of drug is measured by its ability to extend survival time of treated mice and to clear the animals of infection as determined by autopsy and culture of the surviving animals when experiment is terminated. Collaborator has provided some of drugs tested and has synthesized drugs on his initiative or of types suggested by principal investigator.

Part B included.

Major Findings:

One promising new drug has been referred to Laboratory of Clinical Investigations for clinical trial and results of trial have been followed with Dr. Utz and Dr. Andriole.

Significance to bio-medical research and the program of the Institute:

Presently available antimycotic drugs are too toxic and too ineffective for safe and ideal clinical use. Experimental therapy in animals should precede clinical trial.

Proposed Course of Project:

This project will be continued indefinitely.

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Calendar Year 1959

Serial No. NIAID-76-4
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Utz, John P., Treger, Albert, McCullough, Norman B. and Emmons, Chester W. Intravenous Use in 21 Patients with Systemic Fungal Diseases. Antibiotics Annual 1958-1959, pp. 628-634.

Utz, J. P., German, J. L., Louria, D. B., Emmons, C. W. and Bartter, F. C. Pulmonary Aspergillosis with Cavitation. Iodide Therapy Associated with an Unusual Electrolyte Disturbance. N. Eng. J. Med. 260:264-268, 1959.

Emmons, C. W. and Piggott, Willard. Amphotericin B and Griseofulvin in the Treatment of Experimental Systemic Mycoses. Antibiot. & Chemo. IX:550-556, 1959.

Emmons, Chester W. Failure of griseofulvin to control experimental systemic mycoses in mice. A.M.A. Arch. Derm. (In press).

Honors and Awards relating to this project:

None

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-79
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

C. Project Title: Underwater Swimmers Fever.

Principal Investigator: C. W. Emmons

Other Investigators: Dr. Harry A. Horstman, Jr., and Lt. A. J. Kavanagh, Jr., BUMED, Navy Dept.

Cooperating Units: Bureau of Medicine and Surgery, Dept. of the Navy, Washington, D. C.

Man Years (calendar year 1959):

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives:

To determine the etiology of a benign, self-limited, non-contagious febrile illness in underwater swimmers.

Methods Employed:

Isolation of fungi from underwater masks; determination of their pathogenicity and allergenicity by infection and production of hypersensitivity in mice, guinea pigs and rabbits; preparation of sterile antigen for intradermal testing and the diagnostic use of this antigen in field studies.

Major Findings:

Two allergenic fungi have been found in many masks. Their etiologic importance has not been finally determined. We have learned how to prevent the occurrence of the disease by sanitation.

Part B not included.

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-78
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

A. Project Title: Biochemistry and Physiology of Pathogenic Fungi. (In vitro studies of the action of Amphotericin B on pathogenic fungi).

Principal Investigator: Dr. George W. Lones

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: None

Project Description:

Objectives:

To investigate the quantitative relationships of this antifungal drug with respect to fungicidal and fungistatic action; to study certain physical and chemical factors influencing this action; to study the effects of this drug on certain metabolic activities of fungi; to study the emergence of strains resistant to this drug; and ultimately to understand the mechanism of action of this member of a group of related antifungal antibiotics.

Methods Employed:

Candida albicans is used as the test organism. The relationship of drug concentration to death rates, growth inhibition, respiration, and fermentation is studied by standard methods. Antibiotic sensitivities are determined in liquid culture photometrically. The effects of metabolites and related substances on drug sensitivity are determined.

Major Findings:

Due to the time required by Project B no experimental work was performed on this project during 1959. The stock cultures, however, have been maintained in the expectation that the project will be resumed at the first opportunity.

Part B included.

Significance to bio-medical research and the program of the Institute:

Amphotericin B is an antifungal antibiotic receiving considerable attention from clinicians for the treatment of mycoses. Knowledge of its properties, mode of action, and the emergence of strains resistant to it are of importance in the treatment of this increasingly significant group of diseases.

Proposed Course of Project:

It is planned to continue these studies. The study of the quantitative relationships of the antagonism of Amphotericin B by cysteine and the sterols will be elaborated. The resistant variants of C. albicans will be used to study further the mechanism of action of this drug.

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Calendar Year 1959

Serial No. NIAID-78
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Lones, G. W. and Peacock, C. L. Alterations in Candida albicans During Growth in the Presence of Amphotericin B. Antibiotics & Chemotherapy. IX:535-540, 1959.

Honors and Awards relating to this project:

None

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-78-a
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

B. Project Title: Studies on the physiology of Coccidioides immitis.

Principal Investigator: Dr. George W. Lones

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

The biochemistry and metabolism of this dimorphic pathogen have been little studied. It is the purpose of this project to obtain information on the metabolic characteristics of this microorganism, and in particular to discover metabolic differences in the two forms.

Methods Employed:

Shake culture techniques are employed to produce the parasitic and saprophytic morphologic modifications of C. immitis in sizeable quantities for examination by standard chemical and biochemical techniques.

Major Findings:

The method developed for the growth and maintenance of the spherule form of the fungus has been successfully applied for as many as 160 serial transfers. A role of carbon dioxide in the dimorphism of this microorganism has been demonstrated. Studies with the Warburg apparatus have provided information concerning the effect of such factors as pH, prior starvation, and presence of

metabolizable substrate on respiration and fermentation of the spherule and mycelial forms of the fungus. Isotopically labeled glucose has furnished information regarding the proportion of substrate assimilated and oxidized to carbon dioxide.

Quantitative measurements have been obtained of the ability of a variety of potential carbon sources to support the growth of the spherule phase and the mycelial phase.

Significance to bio-medical research and the program of the Institute:

Progressive coccidioidomycosis is a disease with a high mortality. A better knowledge of the causative agent may favorably influence our diagnosis, prevention and treatment of the infection.

Proposed Course of Project:

The examination of the metabolism of the two morphological forms of the fungus will be continued. The nutritional studies will be extended to a greater variety of carbon sources, as well as nitrogen sources in the hope that growth substrates may be found which are more favorable to the parasitic form of the organism than to the saprophytic form.

(Attachment I)

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-78-a
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Lones, G. W. and Peacock, Carl L. Role of Carbon Dioxide in the Dimorphism of Coccidioides immitis, Strain M-11. J. Bact. (Accepted for publication).

Honors and Awards relating to this project:

None

are infected. Studies with candidiasis and cryptococcosis are still in the initial stages.

Significance to bio-medical research and the program of the Institute:

These studies are contributing basic information concerning immunology of the mycoses.

Proposed Course of Project:

The studies with sporotrichosis have been completed and published. Studies with candidiasis and cryptococcosis are in progress.

(Attachment I)

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-79

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Hasenclever, H. F. and Mitchell, William. Attempts to Immunize Mice Against Sporotrichosis. J. Invest. Derm. 33:145-149, 1959.

Honors and Awards relating to this project:

None

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-79.3
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

B. Project Title: Virulence and pathogenic studies with yeasts.

Principal Investigator: Dr. H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	0.8
Professional:	0.3
Other:	0.5

Project Description:

Objectives:

To gain fundamental knowledge with regard to the disease producing characteristics of *Candida albicans*, other Candida species, and *Cryptococcus neoformans*.

Methods Employed:

Comparative virulence studies of strains of *C. albicans* representing extreme ranges of variation in mice or rabbits have been made by the intravenous inoculation of known numbers of cells.

Experiments with *C. neoformans* have been designed to follow the course of progressive disease by determining by plating techniques the number of yeast cells in various organs from soon after inoculation until death of the experimental animals.

Part B included.

Major Findings:

Studies with C. albicans have revealed wide variations in virulence and have indicated that the mouse is as susceptible to candidiasis as the rabbit. These studies indicate that some strains of C. stellatoidea have as much virulence for mice as C. albicans.

The rate of multiplication in vivo of C. neoformans is related to the degree of virulence.

Significance to bio-medical research and the program of the Institute:

These studies are contributing basic information about the variations in strain virulence, and the host-parasite relationship of mycotic diseases.

Proposed Course of Project:

The studies will be continued.

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-79-c
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Hasenclever, H. F. Comparative Pathogenicity of Candida albicans for Mice and Rabbits. J. Bact. 78:105-109, 1959.

Honors and Awards relating to this project:

None

PHS - NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID-79-5
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part A.

C. Project Title: Antigenic Studies on Yeasts.

Principal Investigator: Dr. H. F. Hasenclever

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives:

To learn more about the antigenic interrelationships of some of the provisional pathogens.

Methods Employed:

Rabbits are immunized with yeast cell suspensions, the immune serum collected, and adsorbed with various yeast cell suspensions. Tube agglutinations have been utilized in the antigenic determinations.

Major Findings:

Studies have been made showing the relationship of seven provisional pathogens of the genus Candida and Torulopsis glabrata. Other investigations are still in the initial stages.

Part B included.

Significance to bio-medical research and the program of the Institute:

These studies will contribute fundamental information about specific antigens of the yeast like fungi.

Proposed Course of Project:

To be continued.

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Individual Project Report
Calendar Year 1959

Serial No. NIAID-7[illegible]
1. Infectious Diseases
2. Medical Mycology
3. Bethesda, Maryland

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Hasenclever, H. F. and Mitchell, William O. The Antigenic Relationship of Torulopsis glabrata and Seven Species of the Genus Candida. J. Bact. (In press).

Honors and Awards relating to this Project:

None

PHS-NIH
Summary Statement
Office of Chief
Laboratory of Biology of Viruses
Calendar Year 1959

The Laboratory of Biology of Viruses came into being during the past calendar year by taking the Basic Studies Section of the Laboratory of Infectious Diseases and adding the Unit on Rickettsial Biology and Metabolism of that laboratory.

The primary purpose of creating this new operational laboratory was the grouping together of those research units whose common interests lay in the broad area of the intracellular events involved in viral and rickettsial multiplication as contrasted to research groups with a more disease-directed practical interest in these infectious agents.

In line with the current trend for basic virological studies to be concerned with morphological and biochemical factors both in the virus-infected cell and in the purified virus itself, most of the increased effort initiated in the past year in this laboratory as well as that planned for the next year has been in biochemistry and biophysics. A research associate and a new staff member who is a Ph. D. biophysicist have become associated with the Viral Biochemistry Unit and the Physical Biology Unit will become operational next year. At the same time most of the effort of our Cytology and Metabolism Unit and the Rickettsial Biology and Metabolism Unit have also been directed at the morphological and biochemical aspects of intracellular multiplication of infectious agents.

In spite of the tendency for basic virological investigations to channel into in vitro systems because of their many advantages, we still feel that in vivo studies of virus infections and the impact of immunological, hormonal and other inter-organ mechanisms on the virus-cell relationship are quite important. For this reason, in our Virus Multiplication and Immunity Unit and the Virus-Host Relationship Unit several studies involve virus infection in the natural host animal.

Many of the studies of this laboratory concerned both with the animal cell and the infecting virus attempt to compare the events occurring in tumor cells and normal cells or with tumor viruses versus destructive viruses. The significance of any differences found are obvious.

Although basic in orientation, two of this laboratory's units continue projects of a more practical nature in the fields of immunization against rabies and typhus. This is chiefly due to the background

of experience and continuing interest of the professional people involved but also helps to give the laboratory a feeling of direct immediate application of some of its work.

Significant scientific findings during the past year do not represent any major advance or break-through but rather the beginning of slow, systematic elucidation of individual facets of larger problems and the development of technical and procedural tools for future use. Under the former come the studies indicating two mechanisms of virus-cell association after adsorption, the mode of action of certain virus inhibitors, the intracellular carrier infection with typhoid organisms and their susceptibility to antibiotics, the localization of increased metabolic activity in tumor virus infected cells to perinuclear microsomes, and the resistance of LCM virus carried in tumor transplants to the immune mechanisms in vivo. Technical developments include development of methods for continuous observation of cells in tissue culture by phase contrast microscopy and the better preservation of metabolic activities of subcellular elements.

Serial No. NIAID-20
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Basic Studies of Virus-host Cell Relationships

Principal Investigator: Dr. Karl Habel

Other Investigators: Dr. K. K. Takemoto and Rosalie Silverberg

Cooperating Units: None

Man Years:

Total:	1 4/12
Professional:	1 1/12
Other:	3/12

Project Description:

Objectives:

To determine mechanism of and factors influencing attachment, invasion, multiplication and release of viruses from susceptible and resistant cells.

Methods Employed:

Various strains of tissue culture cells either suspended or on glass are exposed to viruses and at different stages of infection the cells are studied for viability and virus content. All quantitation is by plaque methods.

Major Findings:

Viruses may become associated with cells in a tissue culture system in such a way that they cannot be removed by repeated washing of cells nor by treatment with antiviral antibody and this is true of both susceptible and resistant cells. This cell-association of virus appears to take place by two different mechanisms: One specific and one non-specific.

Part B included: Yes

The eclipse phase of the virus multiplication cycle may take place rapidly in some cells but more slowly in others even though all cells are from an originally cloned culture. There appears to be a stage after adsorption when virus is cell-associated in such a way that it is not neutralized by extra cellular antibody but is not yet eclipsed.

Significance to the Program of the Institute:

These findings suggest specific interaction of virus and susceptible cell at the cell surface which allows virus to become firmly attached and perhaps intracellular before eclipse takes place. If this is true it re-emphasizes the importance of the cell surface as a logical site to apply antiviral effects in any attempts at chemotherapy.

Proposed Course of the Project:

These studies will be continued with attempts to determine morphological and biochemical cellular components responsible for the phenomena already demonstrated.

Serial No. NIAID-80

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Schultz, Edwin W. and Karl Habel. SA Virus--A New Member of the Myxovirus Group. The Journal of Immunology 82: No. 3, March, 1959.

Habel, Karl. The Nature of Viruses and Viral Diseases. The Medical Clinics of North America 43: No. 5, September, 1959.

Honors and Awards relating to this project:

None

Serial No. NIAID-80A
1. Biology of Virus
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Mechanism of oncogenic activity of polyoma virus

Principal Investigator: Dr. Karl Habel

Other Investigators: Rosalie Silverberg and P. Atanasiu (Visiting Fellow)

Cooperating Units: None

Man Years:

Total:	1 11/12
Professional:	8/12
Other:	1 3/12

Project Description:

Objectives:

To determine biological factors involved in the induction of tumors by oncogenic viruses.

Methods Employed:

By studying the virus-tumor cell relationships of polyoma virus in animal and tissue culture systems, the events occurring in new-born versus adult mice and hamsters on injection of virus are being compared. Attempts are being made to induce malignant characteristics in cells in tissue culture and whole tissues in adult animals by varying the physiological state.

Major Findings:

Tumors originally produced by polyoma virus in new-born hamsters have been shown to be transplantable in adults. These tumors apparently are devoid of virus and attempts to "induce" them to activate any possible latent virus state by x-ray and ultraviolet treatment of cells in tissue culture have been negative. Similar virus-produced tumors

Part B included: Yes

in mice have been transplanted in adult mice successfully but here in one series virus remained associated with tumor for 5 transplants.

Significance to the Program of the Institute:

Any information concerning factors responsible for oncogenic properties of tumor viruses may well apply to the oncogenic effects of other agents and provide leads for understanding tumor development under natural conditions in man.

Proposed Course of the Project:

These investigations will continue with the application of any definite finding with the polyoma virus to other proliferative and destructive viral agents.

Serial No. NIAID-80A

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Habel, Karl and P. Atanasiu. Transplantation of Polyoma Virus Induced Tumor in the Hamster. Proc. Soc. Exp. Biol. & Med. 102: 99-102, 1959.

Honors and Awards relating to this project:

None

Serial No. NIAID-302
1. Biology of Virus
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Rabies prophylaxis

Principal Investigator: Dr. Karl Habel

Other Investigators: Rosalie Silverberg

Cooperating Units: Expert Committee on Rabies, World Health Organization

Man Years:

Total:	9/12
Professional:	2/12
Other:	7/12

Project Description:

Objectives:

To improve methods of rabies prophylaxis in man and to reduce non-specific reactions caused by vaccines.

Methods Employed:

Through the WHO Expert Committee on Rabies, various prophylactic schedules with several types of vaccine either alone or combined with antiserum are given to normal humans and the patients bled at intervals. Quantitative determinations of antibody levels are done. The same schedules are tested in animals where immunity to challenge can be performed.

Major Findings:

In a study in 400 normal human volunteers receiving varying inoculation schedules of 3 different types of vaccines with and without an accompanying dose of antiserum the following facts became apparent:

Part B included: Yes

(1) Reduced schedules of vaccine doses below those currently employed in post-exposure immunization are not advisable.

(2) The phenomenon of antibodies in antiserum interfering with antigenicity of vaccine was confirmed and could be overcome by booster doses of vaccine.

(3) Booster doses of vaccine given at 30, 60, 90 or 120 days after varying schedules of primary immunization usually called forth a definite and rapid (within 8 days) antibody booster response. However, not 100% of individuals tested so responded.

(4) Antiserum given at the time of a booster dose of vaccine in persons previously "sensitized" by a course of primary immunization interfered with the booster effect.

In animal experiments it was shown that mice immunized one year previously then given a single booster dose of vaccine at time of challenge showed an increased resistance, thus confirming the results of antibody studies in human volunteers.

Significance to the Program of the Institute:

As a result of these studies specific changes in the procedures recommended for post-exposure and pre-exposure immunization of man against rabies have been made by the WHO Expert Committee on Rabies.

Proposed Course of the Project:

Field studies with WHO will continue. Further investigations in the laboratory will be aimed at methods of eliminating the paralytic factor from vaccines. Basic studies on mechanism of vaccine induced immunity to rabies will be initiated.

Search No. [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Habel, Karl. Prevention of Rabies in Man. Postgraduate Medicine Vol. 25, No. 6, June 1959.

Honors and Awards relating to this project:

None

Serial No. NIAID-81
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epidemiological and laboratory investigations of infectious diarrhea of infancy

Principal Investigator: Rosalie J. Silverberg

Other Investigators: Dr. Karl Habel

Cooperating Units: Dr. Ralph S. Paffenbarger, Robert A. Taft Environmental Health Center, Cincinnati, Ohio

Man Years:

Total	1/12
Professional	1/12
Other	None

Project description:

Objectives:

A continuation of last year's attempt to determine whether causative viruses could be identified with certain epidemic diarrheal diseases of children.

Methods Employed:

In collaboration with Dr. Ralph Paffenbarger of the Taft Environmental Health Center, the children in a small orphanage were studied last year for infectious diseases. Our laboratory has subsequently checked rectal and throat swabs for viruses by tissue culture methods while enteric bacteriological studies were done by the laboratory in Cincinnati.

Major Findings:

228 virus isolations were made from the specimens collected biweekly for one year (222 of these were reported last year). Identification was as follows: 10 Coxsackie B-3, 93 Echo 7, 11 Echo 4,

Part B included: No

75 adenovirus (6 of these were done this year; all the other isolations were reported last year). Of the last named, 49 were Type 1 adenovirus, 1 Type 2, 2 Type 3, 23 adenoviruses, isolated at various times throughout the study year, and not from a single outbreak, could not be typed with the sera available. 39 isolations of a virus, whose properties differ from the known enteric agents were referred to in last year's report; the low antigenicity of this agent has made it impossible thus far to prepare an antiserum for identification purposes.

These studies extending through two years have not demonstrated an etiological role for any of the viruses identified.

Significance to the Program of the Institute:

Many outbreaks and sporadic cases of diarrhea in infants and children, though clearly infectious, are not attributable to specifically identifiable agents. This project was designed to provide information in this area.

Proposed Course of the Project:

Identification of the single group of enteric agents remains to be done, if a suitable antiserum can be prepared. No further specimens have been collected.

Journal No. NIAID-62
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Cell growth inhibiting substances

Principal Investigator: Dr. John W. Hornibrook

Other Investigators: None

Cooperating Units: Drs. Karl Habel and Hilton B. Levy

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

To find and investigate substances and mechanisms which prevent the growth of cells in the adult animal.

Methods Employed:

Isolation from serum and tissues of substances which will inhibit the growth of mammalian cells in tissue culture.

Major Findings:

The inhibiter found last year in guinea pig liver was further purified. It was finally identified as arginase and further investigation of this substance was dropped.

Work was continued on an inhibiter found in serum and ascitic fluid supernatant. Some of these inhibitors are interesting in that they are dialyzable through cellophane.

Part B included: No

Significance of the Program to the Institute:

To determine some of the chemical factors controlling growth is of itself interesting and should be of practical interest to those involved in tissue culture work and cancer research.

Proposed Course of the Project:

Attempts will be made to purify and identify these substances and study their mode of action.

Serial No. NIAID-83
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Mutation in Animal Viruses

Principal Investigator: Dr. K. K. Takemoto

Other Investigators: Dr. Karl Habel and Doris Levinson

Cooperating Units: None

Man Years:

Total:	3 7/12
Professional:	1 1/12
Other:	2 6/12

Project Description:

Objectives:

To isolate, purify and characterize virus mutants and to study these mutants in different virus-cell interactions.

Methods Employed:

Plaque techniques with various virus-cell systems are employed to isolate and purify viral mutants. With this technique, mutants with plaque morphologies different from parental types can be readily isolated, purified by further plaque passages, and studied as genetically homogeneous viral populations.

Major Findings:

(1) An inhibitor against type 1 polioviruses associated with the gamma globulin fraction of certain horse sera was studied. Addition of inhibitory horse serum at low concentrations in overlay medium causes marked reduction in plaque size of certain type 1 polioviruses. Inhibitor-resistant particles capable of forming normal-sized plaques were found in a plaque-purified pool of Mahoney virus. Inhibitor sensitive and resistant type 1 polioviruses have also been found in the stools of patients with paralytic poliomyelitis.

Part B included: Yes

Although the inhibiter is in the gamma globulin fraction of horse serum, it has been found to lack the properties of true antibody. It inhibits viral multiplication primarily by interfering with adsorption and delaying the release of virus from infected cells. The inhibitor is highly specific, it has no effect on plaque development of types 2 and 3 polioviruses, vaccinia, or Coxsackie A9 virus.

(2) By using plaque techniques in a study of Encephalomyocarditis (EMC) virus and mouse fibroblast cells (strain L), two types of particles capable of producing different and distinct plaques have been found. Studies are in progress to determine the basis for the differences in plaque types.

Significance of the Program to the Institute:

Studies on variations and mutations of animal viruses not only lead to further knowledge and understanding of the nature of viruses but have practical implications in the development of vaccines in virus diseases.

Proposed Course of the Project:

The inhibitor which has been isolated in relatively pure form will be further studied in attempts to elucidate mechanisms involved in viral growth. Other genetic properties of the mutants under study will be further investigated.

Serial No. NIAID-83

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Takemoto, K. K. and Habel, K. Virus-cell relationships in a carrier culture of HeLa cells and Coxsackie A-9 virus. Virology 7 (1), 28-44, 1959.

Takemoto, K. K. and Habel, K. Sensitivity and resistance of type 1 polioviruses to an inhibitor in certain horse sera. Virology 9 (2), 228-243, 1959.

Honors and Awards relating to this project:

None

Serial No. NIAID-84
1. Biology of Viruses
2. Virus Host Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Host-Parasite Relations

Principal Investigator: Dr. Victor H. Haas

Other Investigators: None

Cooperating Units: Dr. Hilton B. Levy (NIAID/LBV)
Miss Delta Uphoff (MCI)

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

(a) To determine the nature of the association between the virus of lymphocytic choriomeningitis (LCM) and the transmissible lymphocytic tumor P288, which allows LCM to survive serial passage through immune mice. This includes attempts to make the virus capable of producing tumors.

(b) To study the mouse's immune response to LCM and to alter it in various ways. Based partly on work with LCM-P288 in immune mice and partly on X-ray exposure and marrow transplantation in immune and non-immune mice.

(c) To locate the sites of LCM replication in the infected cell.

Methods Employed:

(a) The transmissible lymphocytic tumor is carried in CDBA mice, with and without accompanying LCM virus. One line, with associated LCM, is maintained in mice immune to LCM. Where indicated, mice are treated with amethopterin. LCM is detected by suitable mouse inoculations.

Part B included: Yes

(b) CBBA mice, some immune to LCM and some not, are given whole body X-ray exposure. They are transfused with isologous bone marrow and spleen from immune or non-immune donors. Survivors are challenged with homologous virus. The marrow transplants are done by Miss Upnoff.

(c) Antibodies are prepared in various animals, conjugated with fluorescing dyes, and used to stain tissues known to contain LCM. Evidence of virus replication as indicated by fluorescence within infected cells, is sought by ultraviolet microscopy. Preparation of fluorescing sera and staining of virus-cell preparations are done by Dr. Levy.

Major Findings:

(a) Last year I reported that LCM virus associated with tumor P288 survived serial passage in mice immune to LCM, if the mice were treated with amethopterin. This year I have adapted a strain of tumor-associated LCM to serial passage through untreated LCM-immune mice. It is now in its 40th passage in untreated immunes. The virus appears to be replicated by the tumor cells and to pass to daughter cells at the time of cell division. This might be considered a form of in vivo tissue culture. It also appears to be analogous to pseudo-lysogeny.

(b) Mice given normally fatal X-ray exposures, then transfused with life-saving isologous bone marrow and spleen from donors immune to LCM, did not become immune as a result of the transfusions. Mice which were immune before their X-ray treatment, and which were saved by marrow from non-immune donors seem not to have lost their immunity. These observations suggest that, in respect to LCM in the mice used, immunity did not depend on or derive from the part of the reticulo-endothelial system represented by bone marrow and spleen. Further studies are in progress, and should provide more information regarding comparative roles of host and graft in response to LCM.

Significance of the Program to the Institute:

The project contributes to two subjects currently of interest to the Institute: (1) Virus-cancer relationships. The serial transmission of LCM in immune mice under the conditions established is the only instance of its kind. It provides an opportunity to determine the effect of virus and tumor upon one another resulting from forced, intimate, and prolonged association. (2) Immune response to infection. The LCM-P288 combination enables the virus to survive despite the mouse's specific immunity. The marrow transplant studies indicate certain limitations of the reticulo-endothelial system in determining the mouse's immune status. In mice, the LCM-host relationship shows some unique features, and these studies should contribute to our basic knowledge of immune response and its limitations.

A third part of the project, dealing with fluorescent antibodies, is of potential significance in respect to virus synthesis but to date the study has been hampered by technical difficulties.

Proposed Course of the Project:

(a) Continuation of studies on the LSH-P288 relationship in LSH-immune mice. Virus removed from these tumor cells will be tested for tumor-producing ability. The condition of the virus in the tumor cells, its relative frequency, its susceptibility to antibodies, and evidence of its synchronization with cell division will be studied.

(b) Marrow transplant studies will be extended to determine the effect upon immune response produced by different combinations of immune and non-immune hosts and transplanted tissues.

(c) Studies with fluorescent antibodies will be pursued along different lines. To eliminate non-specific fluorescence, tissue-culture LSH will be used instead of infected tissues from whole animals.

(d) If there is opportunity, the amethopterin studies reported in previous years will be resumed, but with the dependence on tissue-culture virus growth rather than on infection of the whole animal.

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Individual Project Report
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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Potter, Michael and Haas, Victor H. Relationships between lymphocytic choriomeningitis, amethopterin, and an amethopterin-resistant lymphocytic neoplasm in mice. Jour. Natl. Cancer Inst. 22: 801-809 (1959).

Haas, Victor H. When Bubonic Plague Came to Chinatown. Amer. Jour. of Trop. Med. and Hygiene. 8: No. 2, March 1959.

Honors and Awards relating to this project:

None.

Serial No. NIAID-85
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigations of Animal Virus Reproduction

Principal Investigator: Dr. Milton B. Levy

Other Investigators: Drs. I. Brodsky, Victor H. Haas and Harvey Liebhaber. (For forthcoming year Dr. F. De Filippes).

Cooperating Units: Dr. Victor H. Haas

Man Years:

Total:	3 yrs. 5 mos.
Professional:	1 yr. 5 1/2 mos.
Other:	1 yr. 11 1/2 mos.

Project Description:

Objectives:

To gain information relative to the relationships that exist between the infected cell and the virus reproducing therein. It is anticipated that this program will elucidate some aspects of the mechanism of virus reproduction. In addition, better understanding of the nature of the disease response may be forthcoming from a determination of which of the specific paths of cell metabolism are altered by the disease process. Knowledge of either or both of these phases of the problem (virus reproduction and altered cell metabolism) might be useful in the development of chemotherapeutic agents to inhibit virus growth and to aid the host. Through comparison of a virus causing increased cell proliferation (Friend's leukemia virus) with a destructive virus such as poliovirus, some insight may be gained into growth controlling mechanisms.

Methods Employed:

As in the past we have used many biochemical and biophysical techniques, including spectrophotometry, manometry, paper electrophoresis, paper and column chromatography, radioisotopes, etc.

Part B included: Yes

Major Findings:

Last year's report pointed out that very early in the course of infection with a murine leukemia virus, the spleens of infected mice take up more radioactive phosphorus (P^{32}) per mg. of tissue than do non-infected ones. This year we have confirmed and extended our observations that the intracellular localization of the very early metabolic abberation is restricted to the microsomal and subparticulate RNA and lipid, with no significant changes occurring in the nuclei and mitochondria. After ten days of infection, when the infected spleens are greatly enlarged, all the phosphorus containing fractions studied showed a more rapid metabolic rate than did controls. X-irradiation of animals with advanced disease caused a marked reduction in spleen size of infected animals, with a smaller reduction in size of control spleens. At the same time, P^{32} uptake was decreased in all biochemical fractions, with the greater reduction in infected tissues. This observation is consistent with the idea that the effect of X-ray is on the same aspect of growth common to normal and infected tissue, and not on malignant processes. As would be expected, therefore, the remission is only temporary, and growth soon resumes.

We have suggested (Levy & Brodsky, N.Y. Acad. Sci.) that replication of this virus may involve substitution of viral nucleic acid for normal nucleic acid in the microsome and/or subparticulate components of the cell (which elements are currently credited with playing a major role in protein and possibly ribonucleic acid synthesis). This combined viral RNA would then act as a template for the synthesis of new viral components. Under this hypothesis it might be expected that some fraction of the RNA from these very small cellular components would be more active than the remainder. Ion exchange chromatography studies of infected radioactive RNA has indeed shown this to be true.

One manuscript embodying the first part of the fractionation studies has been submitted for publication, another one is in preparation.

Significance of the Program to the Institute:

We are currently in a period of rapidly increasing understanding of nucleic acid metabolism and biosynthesis. This area is being expanded primarily in the area of straight biochemistry without immediate regard to virus replication or even to tumor growth. This Institute's interest in fundamental virology and from this its derived interest in tumors is well served by strong activity along the lines of application of this biochemical knowledge to viruses. Indeed, comparison of oncogenic viruses with destructive viruses in the metabolic areas discussed above may reveal something about the unique nature of the oncogenic viruses.

Proposed Course of the Project:

With the addition to the unit of two men at the doctorate level (Drs. De Filippes and Dr. Liebhaber) it will be possible to expand the unit's program to encompass types of work not hitherto feasible.

Comparison will be made between the data already described pertaining to the oncogenic Friend's virus in vivo with data to be obtained from a destructive virus in tissue culture.

A number of cell free systems have been described in which suitably fortified fractions from cells can be made to synthesize protein and nucleic acid. Attempts will be made to apply these techniques in such a way that synthesis of virus or virus components might be hoped for. It would be too optimistic to hope for immediate success in synthesizing infective material, so indirect methods will have to be used to demonstrate the formation of new viral components. Such methods are currently under investigation.

We also plan to prepare large amounts of radioactive virus in order better to study its fate on entering a cell.

Serial No. NIAID-85

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Levy, Hilton B., and Brodsky, I. The Effect of a Leukemia Virus on Phosphorus Uptake by Mouse Spleen. Cancer Research 19: 477-484, 1959.

Levy, Hilton B., and Brodsky, I. Some Biochemical Effects of Infection with Friend's Leukemia Virus. Ann. N. Y. Acad. Sci. 81: 51-61, 1959.

Levy, Hilton B., and Snellbaker, L. Phosphorus Metabolism in Infection with a Murine Leukemia Virus. In Press.

Honors and Awards relating to this project:

None

Serial No. NIAID-85A
1. Biology of Viruses
2. Viral Growth
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Simplified method of preparation of gamma globulin for biological studies

Principal Investigator: Dr. Hilton B. Levy

Other Investigators: Drs. Herbert Sober, Samuel Baron and Robert Friedman

Cooperating Units: National Cancer Institute and Division of Biologics Standards

Man Years: (this unit)

Total:	1/12
Professional:	1/24
Other:	1/24

Project Description:

Objectives:

To develop a simple method for the preparation of gamma globulin from serum, and to apply this to a study of antibody development in a variety of human disease states.

Methods Employed:

This project was for the development of a method, and, therefore, methods and major findings are incorporated together in this report.

Major Findings:

The simplified method briefly mentioned last year has been further tested and found effective. A manuscript is in press. Several units at NIH are using the simple chromatographic method described.

Part B included: Yes

Significance of the Program to the Institute:

In the fields of immunology and virology, the availability of a simple method for gamma globulin preparation will enable investigators to eliminate some of the confusion that arises from the use of more complex protein mixtures. This should be particularly valuable in fluorescent antibody studies and in eliminating non-specific inhibitors in various tests such as complement fixation or H. I.

Proposed Course of the Project:

In the future this unit's role in this project will be that of consultant more than that of an active investigator. Drs. Baron, and Friedman of DBS are using further developments of the fractionation method in an attempt to determine which protein fractions of serum contain the several antibodies found during the course of different diseases.

Serial No. NIAID-85A

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Levy, Milton B., and Sober, Herbert A. A simple chromatographic method for separation of gamma globulin from serum. In Press.

Honors and Awards relating to this project:

None

[illegible]
1. [illegible]
2. [illegible]
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigations of interference with enzymatic functioning of mitochondria as a mechanism for carcinogenesis

Principal Investigator: Dr. Hugo L. Hackenbuch

Other Investigators: Dr. Roger W. O'Gara, NCI

Cooperating Units: Laboratory of Pathology & Biochemistry, [illegible]
Laboratory of Pathology, NCI

Man Years:

Total:	7/12
Professional:	6/12
Other:	1/12

Project Description:

Objectives:

To determine whether interference with the functioning of mitochondrial enzymes leads to neoplasia. Specifically, it is desired to find a single dye which: 1) is adsorbed by mitochondria, 2) affects the functioning of mitochondrial enzymes, and 3) induces neoplasia. Then, perhaps, a connection between these two functions of a single [illegible] can be demonstrated.

Methods Employed:

Two of the tumors induced by Light Green, SF, yellowish, and two induced by Fast Green, FCF, have been maintained by transplantation. The metabolism of these tumors has been studied in various transplant generations.

Preliminary studies of localization of the dyes in subcellular elements have been carried out. This was done by mixing the dye to homogenized liver, and then separating the homogenate into different subcellular fractions by centrifugation.

Part B Included: Yes

Efforts are being made to find a method by which significant amounts of the dye, Janus green B, which is known to affect mitochondrial enzymes, can be injected into rats or mice.

Major Findings:

Though it is impossible to distinguish cytologically between tumors of different tumor lines, or tumors of the same line in different generations, there is a pronounced difference in metabolism between them. The early-generation tumors showed barely significant values for glucolysis and respiration. In later generations all four tumor lines showed a much more marked metabolic activity. Two metabolic patterns emerged. One was typical of tumor tissue, i.e., showed higher glucolytic than respiratory activity. The other was closer to that of normal tissue, i.e., did not utilize glucose per se, but utilized fructose-diphosphate and showed marked Krebs cycle activity. Both glucolysis and respiration were shown to be on the mitochondria, when they occurred. Glucolysis also occurred in the supernatant.

Significance to the Program of the Institute:

Both viruses (exogenous) and altered subcellular particles (endogenous) have been implicated as the cause of cancer. This study is an effort to determine the relation of mitochondria to these possible oncogenic agents.

Proposed Course of the Project:

Further studies of the localization of the two triphenyl methane dyes will be made. Studies of their biochemical and biophysical effects on subcellular elements will be made.

Further attempts will be made to induce tumors with the mitochondrial stain, Janus green B.

Serial No. NIAID-86

PHS-WTH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Hesselbach, M. L. and O'Gara, R. W. Fast Green- and Light Green-Induced Tumors: Induction, Morphology and Effect on Host. In Press. J. Natl. Cancer Inst.

Honors and Awards relating to this project:

None

Serial No. NIAID-K[illegible]
1. Biology of Virus[illegible]
2. Virus Host Relati[illegible]
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Demonstration of Glucose Metabolism and Peptide Bond Splitting by Isolated Brain and Liver Mitochondria

Principal Investigator: Dr. Marie L. Hesselbach

Other Investigators: Dr. H. G. du Buy

Cooperating Units: Analytical Chemistry Section, NIAMD

Man Years:

Total:	8/12
Professional:	7/12
Other:	1/12

Project Description:

Objectives:

To demonstrate that isolated mitochondria participate in protein metabolism and that not only isolated brain mitochondria but also liver mitochondria contain the complete enzyme systems to metabolize glucose.

Methods Employed:

Conventional Warburg manometry, chemical lactic acid determinations, centrifugal separation of subcellular elements, biochemical and biophysical approach to choice of materials for a suspension medium which will keep mitochondria structurally and functionally intact, biochemical approach to determining materials to be added to isolated mitochondria to restore *in vivo* enzymatic activity.

For demonstration of peptide-bond splitting: one-dimensional paper chromatography. Ninhydrin spray to demonstrate location of amino acids and peptides.

Part B included: No

Major Findings:

Rat liver is a much better material than mouse liver for this work. Many sugars, including glucose, are readily metabolized by rat liver preparations. Some glucolysis has already been demonstrated on rat liver mitochondria, when individual biochemical materials were added to the reaction vessel.

Glycyl-leucine is split into its component amino acids by all subcellular fractions of mouse brain. Unwashed mitochondria showed good activity, but this was reduced by one washing in sucrose medium. The resultant wash water was also active in splitting the peptide. These findings show that peptidase activity is easily washed off isolated mitochondria. The data support and extend the work of Maver and Greco (JNCI 12: 37-48, 1951), who found the major part of cathepsin activity on the mitochondria of various organs, but only at acid pH's. Both sets of data support the hypothesis that mitochondria are the site of catheptic activity in the living cell.

Significance to the Program of the Institute:

The relation of mitochondria to viruses (site of virus reproduction, origin of viruses, site of neoplastic change) can only be fully understood when their physical and chemical characteristics become known. It would be strange if liver tissue could not metabolize glucose, since it stores it (as glycogen), etc. It would also be strange if this essential, energy-producing metabolism were not located on the mitochondria in liver cells as it is in brain cells.

The work on peptide-bond splitting present additional data on the enzyme complement of mitochondria.

Proposed Course of the Project:

It is desired to obtain liver mitochondria which will readily oxidize glucose in significant quantity. Further studies will be made to obtain better media and to determine what other chemical or physical agents can be added to obtain sustained enzyme activity of a number of complete enzyme sequences on mitochondria in vitro.

Serial No. NIAID-87
1. Biology of Viruses
2. Virus Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Cytopathogenic effect in single cells in tissue culture.

Principal Investigator: Jane L. Showacre

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	6/12
Professional:	5/12
Other:	1/12

Project Description:

Objectives:

To study the morphological and metabolic effects of virus infection on individual virus infected cells in tissue culture.

Methods Employed:

Strain L fibroblasts, HeLa, and other cell lines are grown on coverslips, then placed in a special tissue culture flow chamber which permits direct, phase, darkfield, and fluorescent microscopy under conditions of aeration and medium renewal in order to maintain cells for 24 hours or longer. Cells growing in the chamber are then infected with virus. Changes in morphology and in vital and sub-vital staining characteristics are studied. Still and cine-photomicrographic records are made.

Major Findings:

Studies are in progress with strain L fibroblasts infected with EMC and HA 1 viruses in an effort to elucidate the early effects of viruses on host cells. No gross effects on cell morphology have as

Part B included: No

for been noted during the initial stages of infection. Additional detailed comparisons with uninfected cells are needed and are continuing to be made. Vital and sub-vital dyes, such as acridine orange and Janus green B are employed as tools to detect physiologic differences.

Significance to the Program of the Institute:

Morphological or vital staining evidence of early specific effects of virus invasion of a cell may indicate the locus of virus activity.

Proposed Course of the Project:

Further study of morphological and physiological changes of subcellular elements following the introduction of viruses and other pathogenic micro-organisms.

Serial No. NIAID-07A
1. Biology of Viruses
2. Virus Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Characterization of mitochondria

Principal Investigator: Jane Shaffner

Other Investigators: Dr. H. G. du Buy

Cooperating Units: Dr. W. C. Alford, Chemistry Section, NIAID and
Dr. D. L. Vivian, Laboratory of Cancer
Pharmacology, NCI

Man Years:

Total:	7/12
Professional:	6/12
Other:	1/12

Project Description:

Objectives:

To discover the biochemical nature of the specificity of mitochondrial subvital staining with Janus green B and other dyes.

Methods Employed:

Supernatants from fractionated cells were studied in the presence of the electron transferring dyes, Janus green B and phenazine methosulfate, by manometric, spectroscopic and chemical methods.

Major Findings:

Janus green B and phenazine methosulfate were used to determine the presence of single enzymes, or enzyme systems which catalyze sequential reactions in mouse brain and liver supernatants. Where the absence of functional flavoprotein prevented the use of Janus green B, oxygen uptake in the presence of phenazine methosulfate was a measure of reduced diphosphopyridine nucleotide formed. Thus, it was a measure of the activity of enzymes or enzyme systems, of which

Part B included: Yes

diphosphopyridine nucleotide-requiring dehydrogenases are components. Both brain and liver supernatants were shown to contain enzyme systems which metabolized fructose diphosphate, lactate and malate. Details of these findings are presented in two papers which have been published.

Proposed Course of Project:

The two dyes are being employed as tools in other metabolic problems in this laboratory when suitable, e.g., dehydrogenase detection in fluids, phosphorylation studies. In other respects this project is concluded.

Significance to the Program of the Institute:

Use of the two dyes differentially can supply an index for mitochondrial stability, and can elucidate the complexity of enzymes in biological fluids.

Serial No. NIAMD-87A

PHS-398
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications:

Publications other than abstracts from this project:

du Buy, H. G. and Shoumure, Jane. Enzymes catalyzing sequential reactions in mouse brain and liver supernatant fractions. I. Differential use of Janus green B and pinmazine methosulfate. J. Histochem. Cytochem. 7: 361-369, 1959.

Shoumure, Jane and du Buy, H. G. Enzymes catalyzing sequential reactions in mouse brain and liver supernatant fractions. II. The use of pinmazine methosulfate. J. Histochem. Cytochem. 7: 370-384, 1959.

Honors and Awards relating to this project:

None

Serial No. NIAID-88
1. Biology of Viruses
2. Virus Host Relationships
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biology of mitochondria and its relation to endogenous and viral diseases.

Principal Investigator: Dr. H. G. du Buy

Other Investigators: Mr. M. L. Hasselbach and J. L. Shoemaker

Cooperating Units: Dr. W. C. Alford, Chemistry Section, NIAID

Man Years:

Total	1 5/12
Professional:	11/12
Other:	6/12

Project Description:

Objectives:

Biological definition of normal versus pathogenic or virus-altered mitochondria.

Methods Employed:

The methods encompass the application of the cytochemical findings reported under project No. NIAID-87A. They are also concerned with increasing the efficiency of extracting media for maintaining functional integrity of isolated mitochondria, as measured metabolically. Standard methods for measuring P/O ratios have been used. The sucrose gradient method was used in an effort to separate subcellular particulates of various sizes and densities. The results are applied in tissue culture for long term maintenance of isolated mitochondria.

Major Findings:

Conditions necessary to demonstrate the uptake of inorganic phosphorus accompanying glucose utilization by isolated mitochondria were worked out. Mitochondria and mitochondrial derivatives of

Part B included: Yes

various sizes and densities were separated by the sucrose gradient technic, and by differential centrifugation in ordinary media. It was demonstrated that the same S91 melanoma mitochondrial fractions which metabolized dopa, also metabolized glucose and succinate. This indicates that mitochondria with specialized functions, such as melanin production, also have the basic energy-yielding enzyme systems; or, specifically, that melanin granules are mitochondria.

Significance to the Program of the Institute:

If maintenance of the metabolic function of mitochondria in vitro could be accomplished for periods longer than 6-8 hours, the importance of these elements for the cell could be further shown, and their role in virus multiplication determined.

Proposed Course of the Project:

Further studies of mitochondria from different sources will be carried out in order to learn more about the characteristics of these elements. Additional experiments will be done to explore further the complete enzymatic complement of mitochondria, especially as this relates to synthetic activities. All information obtained will be applied to continued attempts at in vitro cultivation of mitochondria.

Serial No. NIAID-88

PHS-398
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

du Buy, H. G., and Woods, M. W. Segregation of Cytoplasmic Particles in Green Plants. Text presented at: XI International Botanical Congress, Montreal, Canada, August 1959. To be published in: Canadian Journal of Botany.

Honors and Awards relating to this project:

None

Serial No. NIAID-88A
1. Biology of Viruses
2. Virus Host Relationship
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Comparison of the properties of crude and crystallized Coxsackie A-10 virus.

Principal Investigator: Dr. H. G. du Buy

Other Investigators: Mary Steigner

Cooperating Units: Dr. W. C. Alford (Laboratory of Chemical Pharmacology) and Dr. F. B. Brackett (Laboratory of Physical Biology), NIAMD.

Man Years:

Total:	1 3/12
Professional:	1/12
Other:	1 2/12

Project Description:

Objectives:

To obtain preparations of purified crystalline virus and compare its properties with that of crude Coxsackie A-10 virus.

Methods Employed:

Coxsackie A-10 virus of suckling mouse origin is purified and concentrated by chemical, physical and ultracentrifugal means. Purified virus is analyzed for protein and nucleic acid content.

The antigenic activity at various stages of purification is compared with that of crude preparations, using neutralization, antigenic extinction and other techniques. The virus nucleoprotein is chemically compared with the appropriate nucleoprotein of the host (suckling mouse muscle).

Major Findings:

The antigenic activity of purified virus diluted to the same titer as the crude suspension is similar to that of the crude virus

Part B included: No

when injected by the routine method in adult male mice. One ml. of a 1/100 dilution of antiserum obtained by the use of either antigen will neutralize 10^5 to 10^6 infective doses of either crude or purified virus. There seems to be a linear relationship between antigen and antibody at all concentrations studied, indicating that no tissue antibodies are involved.

Significance to the Program of the Institute:

Definitive information concerning physical and chemical properties of purified animal viruses is very meager. Such information is essential to the better understanding of the mechanisms of virus infection.

Proposed Course of the Project:

The virus purification procedure is being improved by the insertion of two new steps: 1) ether treatment for the removal of anticomplementary factors remaining after the original procedure, and 2) cetavlo treatment for removal of certain impurities. Further immunological analysis of the virus at various stages of purification is being carried out. Preliminary studies on the comparison of the nucleoprotein of the host tissue and the virus are in progress.

Serial No. NIAID-89

1. Infectious Diseases
2. Virus & Rickettsial Diseases
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

Calendar Year 1959

Part A.

Project Title: Growth and Metabolism of Rickettsiae

Principal Investigator: Elizabeth B. Jackson

Other Investigators: Hope E. Hoppe, Joseph E. Smadel

Cooperating Units : Cytology and Metabolism Unit, LBV, NIAID

Rickettsial Department, Walter Reed Army Institute of Research

The Commission on Rickettsial Diseases, Armed Forces Epidemiological Board

Man Years (calendar year 1959):

Total: 4 1/3
Professional: 2 1/3
Other: 2

Part B included [X] Yes [ ] No

Project Description:

Objectives

The objectives are: 1) accumulation of information which may lead to eradication of the carrier state in persons convalescent from typhus and typhoid fevers through studies on tissue cultures infected with rickettsiae or Salmonella typhosa and subjected to various experimental procedures; 2) laboratory evaluation of current commercial epidemic typhus vaccine, development of a simpler more rapid potency assay for typhus vaccines and development of a lyophilized stable concentrated killed vaccine.

Methods Employed

The ordinary procedures used in a research laboratory engaged in studies on rickettsial and other microbial agents were employed.

Major Findings

1. Carrier state in typhus and typhoid. The earlier work of this group concerning typhus rickettsiae is summarized in the publications, Nos. 1 & 2, listed in Part B.

(a) Eradication of S. typhosa from infected tissue cultures. Chemotherapy experiments with a number of antibiotics revealed that mixtures of chloramphenicol and streptomycin or chloramphenicol, streptomycin and penicillin would "cure" S. typhosa-infected L cells. Elimination of typhoid bacilli was a slow progressive process requiring 2-3 weeks, usually the latter. Recent experiments with Synnematin B (Abbott Laboratories) have shown this antibiotic to be of greater value than any of the drugs tested thus far. Synnematin, which is structurally similar to penicillin G (differing only in side chain configuration), was tested at concentrations which might be attained clinically. In 13 of 14 experiments tissue cultures were freed of typhoid bacteria over periods of time ranging from 9 to 34 days with a mean of 19 days.

(b) Microscopic observations on S. typhosa in living cells. The extremely long periods of time required to rid cells of S. typhosa by antibiotic treatment as compared to the rapid death of the organisms when exposed to these antibiotics in tissue culture medium in the absence of cells led to a series of direct observations by phase microscopy on intracellular typhoid bacilli exposed to various antibiotics.

Streptomycin (20 ug/ml) acted fairly promptly on intracellular organisms. With 2-3 hours the majority of bacteria had ceased to proliferate although many continued to increase in size for a number of hours. On several occasions the inhibited bacteria were seen to form dense aggregates within the cells. However, even after 5 hours a few of the bacilli continued to multiply at the same rate as bacteria in non-treated cells or in tissue culture medium containing no cells, i.e., about every 30 minutes. Whether such bacteria are actually streptomycin-resistant is not yet know.

Synnematin (20 u/ml) induces spheroplast formation in S. typhosa in vitro, i.e., the cytoplasmic contents are extruded through the bacterial cell wall. Phase observations on infected L cells revealed that within an hour after drug was added intracellular multiplication was inhibited; however, spheroplast formation did not occur, even in 5-6 hours. Moreover, increasing the concentration of antibiotic 10-fold did not enhance production of such changes in organisms. After 19 hours in the presence of 20 u/ml intracellular spheroplasts were observed but many bacillary forms still persisted. The effect of the drug on these extracellular organisms present in the observation chamber was striking; with 20 u/ml of synnematin spheroplast formation occurred in a few of the organisms after 1 hour's exposure and by 3 hours nearly 100% of the bacilli were morphologically altered. Preliminary studies with penicillin (100 u/ml) suggest a similar differential effect on intra- and extracellular organisms.

2. Typhus vaccine. A simplified assay procedure for killed epidemic typhus vaccine was developed and compared with the current standard procedure. In the newer method, mice immunized with dilutions of vaccine were tested for their resistance to a given amount of typhus toxin while the assay currently used determines the

level of antitoxin in the serum of vaccinated guinea pigs. The mouse immunity test provided as accurate a measurement of potency as the guinea pig test and was completed in a shorter period of time.

Procedures for concentrating and purifying rickettsiae contained in infected yolk sacs showed a marked reduction in the amount of normal egg material and gave quantitative yields of immunogenic components as determined by the mouse immunity test. Stability studies on lyophilized preparations of the purified vaccine indicated that when stored at 5°C the protective material was maintained undiminished for at least 17 weeks; storage at 35°C resulted in appreciable loss of immunogenic potency during the 4th to 8th week (see Reference No. 3).

Significance of the Program to the Institute

The carrier state and the resistance of intracellular microbes to antibiotics are among the more important problems facing those interested in the control of infectious diseases.

Proposed Course of the Project

Studies of the carrier state will be continued along the lines developed in the past 2 years. It is anticipated that these will occupy a major portion of the groups' time.

During the coming year work on typhus vaccine will be phased out. Although the subject will continue to be of importance, the establishment of a rickettsial section within the DBS will permit a transfer of efforts.

Serial No. NIAID-69

PHS-NIH

Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Hoppe, H. E., Jackson, E. B., Dasmukhas, J. K., and Smadel, J. E. Study on the growth of rickettsiae. III. Influence of extracellular environment on the growth of Rickettsia tsutsugamushi in tissue culture cells. J. Immunol. 82: 165-171, 1959.

Hoppe, H. E., Jackson, E. B., Dasmukhas, J. K., and Smadel, J. E. Study on the growth of rickettsiae. IV. Effect of chloramphenicol and several metabolic inhibitors on the multiplication of Rickettsia tsutsugamushi in tissue culture cells. J. Immunol. 82: 172-181, 1959.

Smadel, J. E., Jackson, E. B., and Campbell, J. Studies on epidemic typhus vaccine. Arch. Inst. Pasteur, Tunis, 1959. In press.

Smadel, J. E. Rocky Mountain spotted fever, Scrub typhus, Rickettsialpox. Chapters in Cecil, R. L., and Loeb, R. F., (eds.): Textbook of Medicine, 10th edit. Philadelphia, W. B. Saunders Co., 1959, pp. 97-109.

Smadel, J. E. General aspects of viral and rickettsial infections, Hemorrhagic fever, Scrub typhus. Chapters 1, 20, 44. In Rivers, T. M., and Horsfall, F. L., Jr. (eds.): Viral and Rickettsial Infections of Man, 3rd edit. Philadelphia, J. B. Lippincott Co., 1959, pp. 1-10, 400-404, and 869-879.

Smadel, J. E. Status of the rickettsioses in the United States. Ann. Int. Med. 51: 421-435, 1959.

Smadel, J. E. Some aspects of intracellular infections. J. Immunol. 1959 (in press).

Honors and Awards relating to this project:

J. E. Smadel	1959	Bruce Award conferred by American College of Physicians
	1959	Stitt Award conferred by the Military Surgeons of the U.S.
E. B. Jackson	1959	Visiting Scientist, NAMRU 2, Taipei, Formosa (Oct.-Dec.)

LABORATORY OF TROPICAL VIROLOGY

CHIEF's SUMMARY OF RESEARCH PROGRESS

This is the first Annual Report of the Laboratory of Tropical Virology established in January 1959. The laboratory consists of two sections - the Arthropod Borne Virus Section located at the NIH in Bethesda (Head, Dr. William L. Pond) and the Virus Section of the Middle America Research Unit located in the Panama Canal Zone (Head, Dr. Alexis Shelokov). The Canal Zone Section shares its facilities and service personnel with the US ARMY MEDICAL RESEARCH UNIT (MARU), PANAMA of the Research & Development Command US Army (Capt. Osborn L. Taylor, MSC, Commanding Officer). The army unit comprises the Mycology Section of the Middle America Research Unit and thus is under the director of MARU. This creates an unusual situation of a mycology research group submitting its annual report under the serial number assigned to the Laboratory of Tropical Virology!

The first year of the new laboratory has been characterized by growth and maturation of the research program of the component sections. The Bethesda section has developed into a well organized group oriented toward the accomplishment of its two-fold basic mission: 1) to provide competent and prompt assistance and cooperation to the Canal Zone laboratory and 2) to carry on independent research program in the field of arthropod borne virology correlated whenever possible with the investigations of the overseas LTV-MARU staff. The Canal Zone section is depending heavily on this support; it is anticipated that the virus group of the Gorgas Memorial Laboratory will also make increasingly greater use of the facilities offered by the Bethesda laboratory.

During the year the Virus Section of MARU has grown from a field station into a research laboratory engaged in investigations which range from emergency workup of influenza epidemics to controlled laboratory studies of pathogenesis and experimental transmission of encephalomyocarditis virus in guinea pigs. Although the primary emphasis has nevertheless remained on the arthropod borne virus diseases. The staff has applied, modified or developed techniques utilizing most of the diagnostic armamentarium of a modern virus research laboratory, including extensive use of tumoral tissue culture systems, animal and egg inoculation, immune serum production and serological techniques of neutralization, hemagglutination-inhibition, hemadsorption-neutralization and inhibition, and complement fixation. To our knowledge the range of methods and techniques employed is not duplicated in any other laboratory facility in the Americas, outside of the United States and Canada.

The established collaborative relationship between the two virus sections of the Laboratory of Tropical Virology proper will serve well in accomplishment of assigned objectives. In the not too distant future the present director of NARU and possibly one or two of the other members of the Canal Zone laboratory staff will be reassigned to Bethesda. This will necessitate taking a fresh look at a possibility of establishment of a new section of LTV to provide these workers with facilities to conduct research on problems not necessarily related to those under investigation by the staff of the ARBOR Virus section at NIH. This new administrative component might be visualized as a "Section on Tropical Virus Diseases", omitting the exclusive reference to arthropod borne viruses, and could provide support for the NARU group in some additional aspects of ARBOR work, and assistance in non-ARBOR tropical virus studies. (By now it has become apparent that dependence on other laboratories for support in this area is rather tenuous except when specifically indicated for administrative reasons.) Besides providing overseas support, the staff of such a section should be expected to carry on independent creative research in the broad field of tropical virus infections coordinated with the interests of the other two sections in the laboratory.

Attention should be paid to staffing the new Bethesda section in part with clinically trained personnel with a degree of competence in tropical virology to provide a clinical viewpoint and clinical competence not only for the new section but also for the existing ARBOR virus section. This personnel should be mobile and prepared to travel to epidemic areas in Middle America or specifically to the Canal Zone to assist the nucleus of LTV-NARU investigators in epidemic emergencies, extensive epidemiological studies or with the heavy load of high priority laboratory investigations.

The concepts of mobility and interdependence are related to an important policy which should be effected as soon as it is practicable -- rotation of laboratory personnel between the Bethesda and Canal Zone virus sections to provide collaboration and mutual confidence which can not be gained in any other way. The other advantages of a routine rotation policy are obvious.

Because of the unusual physical division and the commitments both in Bethesda and the Canal Zone, LTV needs unusually competent and well rounded administrative support both at home and overseas. So far, non-wholly successful support of the Canal Zone laboratory has been furnished by the Director's office, NIAID. Administrative coordination of the activities of the two virus sections has, however,

be improved by appropriate measures including an integrated administrative office in Bethesda. The mounting responsibilities and commitments of NARU require the highest level of local administrative support. This need is accentuated by the proposed major expansion of the laboratory, reconstruction of the building itself, possibility of housing construction for PHS personnel and the inevitable active growth of the research program into new areas.

The crowding of the Bethesda section's basement space will be considerably relieved with the forthcoming move of the EMC laboratories into the new building, although the space to be allotted will hardly provide facilities for establishment of a third virus section. The original problems in initiating an home base virus laboratory, with many of the assigned personnel having had no previous virological training, have been largely resolved as is evident by several imaginative virological projects now in progress. The recent addition of Mr. Joseph Gibbs supplied the special professional competence in the ARBOR virus field, particularly as related to studies of viruses which constitute a health hazard to the personnel. It is assumed that physical facilities to permit regular operations involving use of hazardous agents will be a part of the Bethesda section's permanent space.

Undoubtedly, the Canal Zone virus laboratory would be strengthened if the two currently proposed projects are activated in the future. Dr. James Bauman of the Rocky Mountain Laboratory has discussed interesting and realistic plans for a study of role of bulgaws in transmission of tropical virus infections which would necessitate additional service and office personnel, space and facilities. Dr. Vernon Knight, Clinical Director of NIAID, is considering a challenging possibility of establishing a unit of the Laboratory of Clinical Investigation on the isthmus to be devoted to thorough clinical study of patients with selected tropical diseases. The impact of such a program, while most stimulating and desirable from the standpoint of NARU's staff, would undoubtedly create new and complex problems the solution of which would require not only funds but considerable administrative skill and acumen.

Some of the current research program of the Canal Zone virus section will suffer with the departure of Dr. John Craighead in June 1960. During the past year Dr. Craighead has assumed responsibility for a number of important aspects of the laboratory activities. It is not likely that it will be possible to replace him with a young physician trained in virological methods and with epidemiological and/or ecological inclinations. Fortunately, Dr. Ferebee and

Dr. Vogel have developed here excellent [illegible] many of the phases of the [illegible] position gradually to assume responsibility for [illegible] procedures. Drs. Brady and Lehrer have [illegible] techniques and are already [illegible] intermediate sub projects in [illegible] program.

[illegible] with Dr. Tyler's function [illegible] Valley Lab. [illegible] massive [illegible] logue fibre [illegible] the [illegible] the physical [illegible] Americium [illegible]

[illegible] practice, and [illegible] orthopod cores [illegible] can [illegible] and heterologous [illegible] of the phenomenon [illegible] reactive sera against [illegible] available [illegible] quantitation and [illegible] newly observed [illegible] by [illegible] [illegible] [illegible]

of them of a virus quite unfamiliar in its biological characteristics; 2) the 18 months' study of the eastern equine encephalitis virus in Panama resulted in elucidation of the role of this virus infection in horses and human beings in Panama and disclosed the presence of antibodies evidencing infection not only in certain birds and mammals, but also in reptiles; 3) the encephalomyocarditis virus has been found to be the cause of a fatal disease in swines; its possible pathogenesis and virus isolation are being investigated; this serves as the first conclusive demonstration of this virus in Central America; the finding may lead to the establishment of a new important zoonosis; 4) interesting results are being accumulated by examination of the bi-weekly collections of rectal swabs from Panamanian infants under 3 years of age; this longitudinal study has disclosed a periodic fluctuation of certain enteroviruses related and unrelated to community illness; 5) epidemic influenza outbreaks in Panama (Type B), in British Guiana (Type A_2) and in Costa Rica (Both A_2 & B) were investigated and laboratory support of the Costa Rican national program of live polio vaccine administration was provided.

Ecology Section, NAMRU. 1) During the year approximately 50 cases of clinical histoplasmosis were confirmed by serological techniques and 2 fatal cases of disseminated histoplasmosis were confirmed by recovery of the organism from clinical materials (these were in native Panamanians and represented the second reported fatality in a child and the first in an adult). 2) Epidemiological investigations of all confirmed clinical cases of histoplasmosis resulted in the first isolation of H. capsulatum from Panamanian soil around a dwelling in which two afore-mentioned occupants developed clinical disease. 3) The ecology and microecology of H. capsulatum remains unsolved; however, the organism has been repeatedly isolated from fresh soil during both dry and wet seasons. Naturally occurring histoplasmosis has been demonstrated in guinea pigs and opossums by isolation of the organism from tissue inoculums. These infections were accompanied by diagnostic skin and complement fixation titers. 4) The approaching completion of the bi-weekly enteric and tuberculin skin testing program in the Canal Zone school children will furnish data on approximately 40,000 children between the ages of 6 and 15 years. The current preschool age program will concentrate on the younger age groups.

In summary, it should be recorded with satisfaction by all concerned with the establishment and operation of the Laboratory of Tropical Virology and the Middle America Research Unit that the unusual and complex arrangements have worked out remarkably well. With provision for additional administrative support for the present three sections, the program will be strengthened to allow further expansion of the overseas facility in terms of not only space and possibly personnel, but most

Control No. NAMRU-3
1. Technical Memorandum
2. Virus Section, Middle America
Research Unit
3. Project Annual Report

Middle America
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of Arthropod Borne-Virus Diseases and their Epidemiology and Ecology in the American Tropics.

Principal Investigator: C. Alexis Sheloha

Other Investigators: Dr. John Craighead, Dr. Charles Dobrovolny, Dr. Joseph Brady, Dr. Pauline Parodis, Jean Hayes and Dorothy Longfellow

Cooperating Units: Arbovirus Group (Yale Section, CVV, Dr. Charles Mitchell Laboratory, N. de P. Pan American Sanitary Bureau, PHS/AMO U.S. Army Biotic Control and Survey Section U.S. Army Mission to Panama Laboratorio Diagnostico de Investigation Enfermedades, N. de P. Chief Surgeons for the Army, Navy and Air Force the U.S. Embassy Clinics Ministry of Health, N. de P.

Man Years (Calendar year 1959):

Total: 6½
Professional: 3
Other: 3½

Project Description: See modified summary of the studies and appropriate entries in the table of contents of the recommended portions of "Studies of Arboviruses of the Middle America Research Unit, April 1958 - September 1959" prepared on 25 September 1959 for the members of the Ad Hoc Committee on Field Evaluation.

Part B included: Yes No

Serial No. MIAID - 9.
1. Tropical Virology
2. Virus Section, Middle America Research Unit
3. Panama Canal Zone

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of Enteric and Respiratory Virus Diseases in the American Tropics

Principal Investigator: Dr. Alexis Shelokov

Other Investigators: Dr. John Craighead, Dr. Jacob Brody, Dr. Paulino Peralta, John Vogel and Dorothy Longfellow

Cooperating Units: Pan American Sanitary Bureau, PAHO/WHO
Gorgas Memorial Laboratory, R. de P.
Ministry of Health R. de P.
Hospital del Nino, R. de P.
Ministry of Health, Costa Rica
Health Bureau, C.Z.
Department of Health, British Guiana
U.S. Army Mission to Panama
Gorgas and Coco Solo Hospitals, C.Z.
Universidad del Valle, Cali, Colombia
Division of Biologic Standards, NIH
Chief Surgeons for the Army, Navy and Air Force of the U.S. Caribbean Command

Man Years (Calendar Year 1959):

Total: 4½
Professional: 2
Other: 2

Project Description: For detailed summary of the studies see appropriate entries in the Table of Contents of the appended portion of "Review of Activities of the Middle America Research Unit, April 1958 - Sept 1959" prepared on 15 September 1959 for the members of the Ad Hoc Committee on MARU Evaluation.

Part B included Yes No

Serial No. [illegible]
1. Project [illegible]
2. Funding Agencies: [illegible]
American Research Units
3. Panama Canal Zone

[illegible]
Individual Projects Report
Calendar Year 1965

Part II.

Project Title: Studies of Mycotic Diseases in the American Tropics with Emphasis on Histoplasmosis

Principal Investigators: Dunn, Colbert L. Taylor, M.D., U.S. Army

Other Participating Personnel: Gray, Herbert L. Kaufman, M.D., U.S. Army; Castellani, [illegible]

Cooperating Units: Mycology Section, Department of Bacteriology, Walter Reed Army Institute of Research
Gorgas Memorial Laboratory, Dr. Carl
Pan American Sanitary Bureau
Chief Surgeons for the Army, Navy and Air Forces of the U.S. Representation
U.S. Army, Caribbean Medical and Dental Units
Health Bureau, Canal Zone
Gorgas and Coco Solo, Hospitals, C.Z.
Santo Tomas Hospital, Panama City
Hospital del Niño, Panama City
Military and Transport Services

Year Started: Calendar Year 1955

Total Man-Years Professional: 1
Other: 1/2

Brief Description: For complete summary of the studies (as appeared in article [illegible] Table of Contents of the appendix [illegible] or "Review of Activities of the Tropical Medical Unit, Gorgas Memorial Laboratory, September 1962" prepared on [illegible] 1963 for the members of the Ad Hoc Committee on [illegible] Evaluation.

Publications: Included Yes No

Section as (1)-2:
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

I. A.

Project Title: Studies on tropical arthropod-borne viruses in tropical and subtropical areas of the United States and Middle America

Principal Investigator: Robert H. Pennington

Other Investigators: William L. Reed
Clarence J. Gibbs

Cooperating Units: Pan American Sanitary Bureau
Ministry of Health, Guatemala
University of Miami School of Medicine
Southwest Blood Banks, Inc.
Gorgas Memorial Laboratory, Republic of Panama

Man Years (calendar year 1959):
Total: 2-3/4
Professional: 1-1/4
Other: 1-1/2

Project Description:

Objectives:

Serological studies of sera obtained from Guatemala, Panama, and the Southern United States were planned (and have been accomplished in part) in order to afford guidelines for the interpretation of future serological tests whenever new tests are used in an aid in the diagnosis of diseases of man in the respective geographic areas. Moreover, these serological tests were expected to give an indication in a rough way, of which arthropod-borne viruses could be present and which viruses are probably absent from the above-mentioned tropical and subtropical areas of the Western Hemisphere.

Methods Employed:

Neutralization, hemagglutination inhibition, and complement fixation tests of arthropod-borne viruses are being carried out on sera obtained by other investigators from residents of Central America and Southern United States.

Major Findings:

The serological test results of sera collected from human beings in eight areas of Guatemala and six areas of the Republic of Panama and the Canal Zone indicate that at least three Group B viral agents may be active in Guatemala and Panama, i.e., St. Louis encephalitis, Ilheus and Yellow Fever viruses. It is realized, however, that in the absence (thus far) of comparable serological tests for dengue inactivity (or other Group B reactivity), one cannot exclude the possibility that the Ilheus and St. Louis encephalitis reactions may be associated with other antibodies of the B Group. The Yellow Fever antibodies cannot, in these tests, be interpreted except as an indication of probable previous infections with Yellow Fever, either from natural infection or infection induced by vaccination.

The serological test results of sera from Miami residents indicate the activity of St. Louis encephalitis, encephalomyocarditis, and possibly even Ilheus virus.

Significance to Program of the Institute:

The potential and actual importance of the tropical arthropod-borne viruses as a cause of infection of residents of Central America and the Southern United States should be determined. This knowledge is a prerequisite to the study of tropical arthropod-borne viral diseases.

Proposed Course of Project:

It is intended that neutralization, hemagglutination inhibition, and complement fixation tests will be carried out on sera not already tested and the serum collections on hand will be augmented with additional sera from the same areas and areas in nearby countries.

Part B included - No

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of arthropod-borne viruses in tissue culture. Part I. Evaluation of tissue culture systems for use in virus isolation, identification, and in serological tests for viral antibodies

Principal Investigator: Charles R. Rosenberger

Other Investigators: Walter L. Newton

Cooperating Units: Laboratory of Germfree Animal Research, NIAID-118

Man Years (calendar year 1959):
Total: 2
Professional: 3/4
Other: 1-1/4

Project Description:

Objectives:

This study is intended to seek out the most sensitive tissue culture cell lines for isolation techniques, to develop a system for virus identification, and to establish suitable tissue culture techniques for serological tests, all in relation to arthropod-borne viruses.

Methods Employed:

A variety of cell types are being tested for sensitivity to arthropod-borne viruses as determined by evidence of cytopathogenic effect, infectivity titers, and degree of propagation of the virus. The decline of activity of the virus under varying conditions without host cells is determined and compared with action of the virus in tissue culture systems. Particular emphasis is placed on determining adequate tissue culture systems for arthropod-borne viruses which are not readily detectable in tissue culture. Several passages of viruses in tissue culture are compared with mouse brain passaged viruses or viruses recently isolated from natural hosts. Various techniques are employed to determine the activity of the viruses in tissue cultures. These techniques include observations for cytopathogenic effect, hemadsorption tests, formation of plaques, microscopic examination of stained cells for inclusion bodies, and serial passages in various tissue cultures and animals.

Major Findings:

Results of the evaluation of the susceptibility of many tissue culture cell lines and the activity of many arthropod-borne viruses using some fifteen different cell lines have shown hamster kidney cell cultures to be outstanding in serving as a culture medium for many viruses. More intensive investigations of a group of nine viruses in hamster kidney tissue culture has shown that on continued passage these viruses lose much of their pathogenicity for mice. A detailed account of this work has been prepared and submitted for formal publication in the near future.

During the course of these investigations and in collaboration with Dr. Walter L. Newton of the Laboratory of Germfree Animal Research NIAID, kidney tissue cultures from "germfree" mice were prepared and inoculated with various arthropod-borne viruses. Our preliminary results indicate that a greater cytopathogenic effect is produced by dengue Type 1 virus (Hochiwki strain) in germfree mouse kidney cells than in mouse kidney cells prepared from conventional mice.

In investigations of the possibility of undetected viruses in "germfree" animal colonies, no virus could be isolated when tissues were tested in various tissue culture lines and in mice. Certain mouse kidney cell cultures prepared from "germfree" mice have shown some changes suggestive of virus activity. In passages made from these cultures it has been difficult to maintain virus activity beyond three successive passages in tissue culture.

During the course of routine work with hemadsorption techniques in hamster kidney tissue culture a new phenomenon, hemonuclear adsorption, was observed when erythrocytes from one day old chicks were added to hamster kidney tissue cultures which had been inoculated with related viruses. Essentially this reaction results in disintegration of the cytoplasm of the chick erythrocyte leaving the free nuclei which are adsorbed to the infected tissue culture cells.

Significance to the Program of the Institute:

It is hoped that these studies of arthropod-borne viruses in tissue culture will indicate not only the optimal methods for primary viral isolations but also will indicate the best virus-host cell systems for laboratory study of established viral strains.

Proposed Course of the Project:

Studies will be continued along lines which contribute to the basic objectives outlined. Important leads bearing relationship to these projects will be pursued as they arise.

Part H Included - No

Serial No. NIAID-94-B
1. Tropical Virology
2. Arthroped-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part I.

Project Title: Studies of arthroped-borne viruses in tissue culture. Part 2. Development of a tissue culture system utilizing arthroped tissue

Principal Investigator: Charles R. Rosenberger

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1/4
Professional: 1/4
Other: None

Project Description:

Objectives:

To develop a practical tissue culture of arthroped cells which would provide a sensitive and more suitable culture medium for physiological studies of arthroped-borne virus cell-host interactions.

Methods Employed:

Specific tissues are dissected from insects, washed repeatedly in culture media containing antibiotics and placed in culture slides or tubes. The blood of the insect may be collected and added to the culture media. Various culture media and culture techniques are used in attempts to provide a culture system suitable for a particular tissue. Arthropods are occasionally reared under sterile conditions so that the entire animal may be used in culture without contaminating organisms.

Major Findings:

In attempts to culture insect tissue some cells have remained alive for several weeks and in certain instances some apparent growth was observed; however, practical arthroped tissue culture has not been established.

Significance to the Program of the Institute:

Tissue culture of arthropod cells would provide a completely new approach for physiological studies of viruses, host cells, and their interactions. In the field of arthropod-borne virus research, insect tissue culture may provide a sensitive and more suitable culture medium.

Proposed Course of the Project:

Basic studies in the field of tissue culture of arthropods will be continued.

Part B included - No

Serial No. NIAID-95-A
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on antigen-antibody reactions of the arthropod-borne viruses. Part I. Investigations of the comparative rates of reaction of arthropod-borne viruses with homologous and heterologous neutralizing antibodies

Principal Investigator: Robert M. Pennington

Other Investigators: William L. Pond

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To investigate the arthropod-borne virus neutralization test as a dynamic occurrence. Specifically, the variables of the neutralization test being included in the investigations are time (as an independent variable) and the relative degree of reaction (amount of neutralization). Information from this approach is expected to give insight into the actual kinetics of the antigen-antibody combination and reaction which we usually measure as neutralization of antigen virulence.

Methods Employed:

The rapid *in vitro* combination of antigen with homologous antibody and the comparatively slow speed of reaction (avidity) with heterologous antibody is being successfully applied to markedly increase the specificity of the arthropod-borne virus neutralization tests. Project-type serological tests, results of which are likely to be uninterpretable because of broad cross-reactive antigenic relationships, are being studied by kinetic neutralization studies.

Major Findings:

Neutralizing indices of St. Louis encephalitis antiserum against

St Louis encephalitis virus without incubation and following 2 hour incubations is seen to be 2.6 and 3.0 logs respectively, a difference of only 0.4 of a log. This data seems to indicate that the neutralization of virus by homologous antibody is an instantaneous phenomenon. However, Ilheus antiserum, when tested for neutralization of St. Louis encephalitis virus, had a neutralization index of only 0.6 of a log when tested without prior incubation (0 time) and 1.6 logs if incubated 2 hours prior to incubation into experimental animals. This was interpreted as evidence that the degree of neutralization of virus (antigen) by a closely related heterologous antibody increases as the incubation time is increased. This hypothesis was further strengthened using the JE and WN virus-antisera systems.

Significance to Program of the Institute:

The kinetic neutralization studies present a means for further increasing the specificity of the mouse neutralization test. For the first time in broad serological survey work carried out at Laboratory of Tropical Virology, and other institutions, a means of identifying a specific antigenic stimulus is possible where there once existed a confusing broad antibody profile. Moreover, this study also demonstrates that the basic phenomenon of instantaneous homologous antigen-antibody combination existing in bacterial antibody systems also function in the same manner with virus antibody systems.

Proposed Course of the Project:

The concept of "instantaneous" neutralization of viruses with homologous antibody is now being tested using broadly reactive serum specimens obtained previously from indigenous Southeast Asian patients while in the acute and convalescent phase of some Group B arthropod-borne viral infections. The specific causative agent has not been determined because of the extreme Group B interrelations in sera from residents of this geographic area.

Part B included - No

Serial No. NIAID-03-B
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on antigen-antibody reactions of the arthropod-borne viruses. Part 2. Development of a practical and specific flocculation test for the demonstration of arthropod-borne virus antibodies

Principal Investigator: Robert M. Pennington

Other Investigators: William L. Pond

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/2
Other: None

Project Description:

Objectives:

Development of a rapid flocculation test for the detection of arthropod-borne virus antibodies and its evaluation as a worthwhile laboratory procedure.

Methods Employed:

Arthropod-borne viruses, in the form of infectious tissue culture supernatant fluids, are adsorbed onto clay particles in the manner previously described by Bozicevich of the Laboratory of Immunology for the Bentonite Flocculation Test for Trichinosis and for other diseases. In the same manner also, specific flocculation of the antigen-coated Bentonite particles is demonstrated when in the presence of specific viral antibody.

Major Findings:

The Bentonite Flocculation Test has recently been applied in our Section to the antigen-antisera systems of St. Louis encephalitis, Eastern equine encephalomyelitis, Sindbis, and dengue Type 2 viruses with apparent preliminary success. Satisfactory viral antigens, intended for subsequent adsorption onto clay particles, were prepared from virus suspensions after propagation in hamster kidney

tissue culture systems.

Although the nature of the antibody being measured by this test is, as yet, undetermined, the results of a few tests indicate some correlation between the presence and titer of neutralizing, complement fixing, and hemagglutination inhibiting antibodies and the Bentonite Flocculation Test antibodies. The few observations on the specificity of this reaction indicates that it is as specific, if not more so, than the neutralization test.

Significance to the Program of the Institute:

The Bentonite flocculation techniques, as applied to the arthropod-borne viruses and their antibodies, will constitute another valuable serological test to be used in conjunction with the well-known complement fixation, hemagglutination inhibition, and neutralization tests. Moreover, preliminary observations also indicate that the antigen-Bentonite combination is applicable as a simple rapid method for concentration of virus from suspension and may be of value in subsequent adsorption of selected antibody from serum.

Proposed Course of the Project:

Evaluation of the Bentonite Flocculation Test (as applied to arthropod-borne virus systems) in terms of practicality for use in the field; in terms of specificity, the sensitivity, and the general nature of the antibody being measured; in terms of the interpretation of the results obtained from the test; in terms of its applicability to the diagnosis of disease; in terms of the reproducibility of the results and of the stability of the test reagents; in terms of possible improvements in the methods employed for preparing the test reagents and carrying out the test.

Part B included - No

Serial No. NIAID-96-A
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Transmission of arthropod-borne viruses by mosquitoes. Part 1. Pathogenesis of Ilheus virus in adult and suckling mice

Principal Investigator: Herbert T. Dalmat

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To determine the virus-host relationships of tropical arthropod-borne viruses, using Ilheus fever virus as a model. Determine appropriate experimental host animals for laboratory studies, as shown by viremias developed and infectivity of the animals for arthropods; and by effect of virus dose, route of inoculation, and age of host on viremia and disease.

Methods Employed:

These studies are carried out by means of infecting various species of animals, and also inoculating different aged animals of the same species with varying doses of virus by different routes and determining, by titration of blood in 24-day-old mice, the amount of circulating virus developing at intervals after infection. Serial passages were made at predetermined intervals in attempts to establish virus strains in a particular animal.

Major Findings:

Chicks are unsuitable as laboratory hosts for Ilheus transmission studies since the proportion of those inoculated that subsequently develop viremia is too variable and those that did develop viremias were non-infective for mosquitoes.

Arthropod transmission of Ilheus virus can be consistently successful if newborn to 8-day-old mice are employed as host animals, and these are best inoculated by the intracerebral route.

Although there is no substantial difference in susceptibility to Ilheus virus in infant and adult mice, there is a marked difference in the development of virus they circulate in their blood.

The amount of virus in the brains of both 3-day-old and 21-day-old mice increases up until 88 hours following inoculation, decreasing thereafter. Since mice usually die 5-6 days after inoculation, perhaps in attempts to obtain maximal amounts of virus routinely infected mouse brains should be harvested 1-2 days prior to the first appearance of signs of disease, as exemplified by a few other viruses such as the Lansing strain of poliomyelitis virus when used to produce infection in mice.

The presence of virus in the lungs of 3-day and 21-day-old mice and the apparent correlation of quantity of virus in the lungs with that in the blood, suggests the possible "spill-over" of virus from the blood to the lungs.

The subcutaneous route of inoculation of Ilheus virus into mice probably is the closest of the usual routes used to that of mosquito transmission by its bite, and results in viremias about equivalent in concentration of virus to that produced after intraperitoneal inoculation, but lower than that following intracerebral inoculation.

Significance to the Program of the Institute:

With our knowledge of the presence of certain arthropod-borne virus diseases in the United States, and the possibilities that others might actually be present or might be introduced from countries to the south, as much as possible must be learned about these diseases, such as which animals might serve as reservoirs, what conditions must be satisfied for transmission to take place, and all other characteristics of the disease. The studies being carried out with Ilheus virus will serve as a model for similar studies of other arthropod-borne viral diseases.

Proposed Course of the Project:

Initiate similar studies with other arthropod-borne viruses. Repeat a limited number of the studies, using a strain of Ilheus which has not been successfully passed numerous times through mice by the intracerebral route, but rather has recently been isolated from mosquitoes in the field.

Part B included - No (Included for this principal investigator in Project Serial No. NIAID-96-B)

Serial No. NIAID-96-E
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Transmission of arthropod-borne viruses by mosquitoes. Part 2. Transmission studies on Ilheus virus using mice as hosts and Aedes aegypti as vectors

Principal Investigator: Herbert T. Dalmat

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To determine the interrelationship of mosquitoes and the tropical arthropod-borne viruses; determine relation of circulating virus concentration in the blood of hosts to infectivity for mosquitoes; determine relationship of duration of virus incubation in the mosquito to the ability of the mosquito to pick up and transmit virus; determine shortest interval after an infectious meal at which a mosquito can transmit virus; where in the insects the virus lodges or multiplies; techniques for better recognizing the presence of virus in mosquitoes.

Methods Employed:

At different intervals after suckling mice were infected, viremias were determined and pools of mosquitoes were permitted to feed on the mice. After appropriate incubation periods, the mosquitoes were tested for their ability to transmit by bite to susceptible mice and also for virus content in their body.

Large numbers of mosquitoes that had previously fed on mice with known viremias were individually refed each day on susceptible mice which were thereafter observed for death and tested for presence of virus.

Major Findings:

Although investigators subsequent to the original isolation of Ilheus virus have been unable to transmit the virus by mosquito bite from animal to animal, we were able to achieve transmission by Aedes aegypti at 7 days after their infectious meal as well as after 12-14 days. Transmission was especially successful when the circulating virus titer in the donor mice was above 4 logs LD_{50}. The number of mice dying after bites by infected mosquitoes was neither an all or none result and was reminiscent of what would be expected if one LD_{50} were to be inoculated, a situation quite difficult to reproduce even with known inocula.

Ninety-eight percent of mosquitoes used in the large study were able to transmit sometime between 2-14 days after their infectious meal while only in 38 percent were we able to demonstrate virus in their bodies by triturating and inoculating body suspensions into mice on days 17-20. Most of the mosquitoes (92%) were able to transmit by bite by day 9 and more were transmitting on days 7-9 than on days previous to or after that interval. In another experiment, the infection rate in the mosquitoes was relatively high throughout, while very few mosquitoes actually transmitted by bite. The viral content of a single intact mosquito appears to increase sometime between 7-10 days after its infectious meal, which corresponds with the period of maximum demonstrable infection rate.

Significance to the Program of the Institute:

Same as for project (Serial No. NIAID-96-A) on "Pathogenesis".

Proposed Course of the Project:

Continue with some of the objectives already stated. Initiate similar studies with other arthropod-borne viruses of the tropics. Determine the most efficient number of mosquitoes to use for consistent returns in studies of transmission by bite and in preparing pools for isolation of virus. Determine virulence of virus in dead mosquitoes since in field survey work, mosquitoes are often dead by the time virus inoculations are attempted. Repeat a limited number of the studies, using a strain of virus closer to original isolation, to see if the transmission pattern differs from what has been determined with a strain that has already undergone 3f mouse-brain passages.

Part B included - Yes

FWS-HHH
Individual Project Report
Calendar Year 1959

Item 1. Honors, Awards, and Publications

Publications other than abstracts from this project:

Nelson, H. A.: Arthropod transmission of rabbit fibromatosis (Shope). J. Hyg. 57: 1-30, 1959.

Nelson, H. A. and Scanlon, M. F.: A comparative study of the Shope fibroma in rabbits in relation to transmissibility by mosquitoes. J. Nat. Cancer Inst. 22: 593-615, 1959.

Honors and duties relating to this project:

Dr. Robert H. Nelson was elected a Trustee of the Thomas Say Foundation, Entomological Society of America (1958 to date).

Serial No. NIAID-97
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Effects of artificially varied environment on potential mosquito vectors of arthropod-borne viruses

Principal Investigator: Paul A. Woke

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

To provide a basis of methods and biological data for planned studies of virus-vector-environment interrelationships, field epidemiological and control investigations of arthropod-borne virus diseases, and of means for maintaining Haemagogus equinus under optimum conditions for use in experimental investigations.

Methods Employed:

Haemagogus equinus, a known mosquito vector of yellow fever, has been studied under varied environmental conditions in the field and laboratory and its responses measured. On the basis of analyses of the results of these studies, methods and equipment have been modified, and new equipment has been devised to improve laboratory culture conditions. Determinations were made of the survivorship distribution under the improved conditions of culture.

Major Findings:

Means were found which greatly improve the culture methods for maintaining Haemagogus equinus. This was accomplished principally by the design, on the basis of biological studies, of new culture equipment which eliminates many of the outstanding conditions that lead to disorders and premature deaths, and which provides more effective conditions for mating, feeding on carbohydrates and on

animals for blood, and for ovipositing, and with greatly increased ease in care and reduced opportunities for escape of the mosquitoes. Environmental conditions were improved by determinations of the more nearly optimum conditions and their provision within the limits of the equipment available. The longevity of caged adults of Haemagogus equinus has been greatly extended by improved methods of culture, females now living up to 94 days, males up to 107 days, in contrast to previous maximum of 69 and 64 days respectively.

Significance to the Program of the Institute:

The results of these studies help to prepare a sound basis for: (1) field studies of survival in the natural habitat and laboratory analytical studies of internal (physiological and pathological) factors in survival; (2) laboratory maintenance of the species under optimum conditions for maximum effectiveness in research, so that (a) results of experimental work may be expected to reflect the potentialities of the species uninfluenced by effects of an inferior environment, (b) the possible selective effects of conditions adverse to the population may be avoided, and (c) standardization of strains for experimental purposes may be accomplished; (3) field epidemiological investigations of arthropod-borne virus diseases; (4) laboratory investigations of virus-vector-environment interrelationships; (5) investigations directed toward the development of control measures against arthropod vectors of virus diseases.

Proposed Course of the Project:

Through studies of the vector in the laboratory and in the natural habitat, using the improved means for maintaining and studying the vector, and with adequate control over the production and management of the conditions known to effect the health and survival of vectors, to determine the approximate survival potential of the species under controlled constant uniform environmental conditions within the optimum range.

Similarly, but in order to more closely approach natural conditions, to study behavior and survival under controlled environmental conditions that are varied through a diurnal cycle approximating usual cycles found in the natural habitat.

To make similar studies, but in which differentials in intensity of different environmental conditions are provided in different parts of the culture chamber.

To apply the findings of these studies and extend the studies to include first generation Haemagogus equinus and to include vector species other than Haemagogus equinus.

To apply the findings to studies in the interrelationships of viruses, vectors, and the environment.

The above proposed studies require equipment which has been requested but is not at present authorized by this Institute for purchase. The equipment currently in use is unsuited for producing scientifically reliable results and lacks essential versatility.

Part B included - No

Serial No. A [illegible]-3
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on tropical arthropod-borne viruses in tropical and subtropical areas of the United States and Middle America

Principal Investigator: Robert M. Pennington

Other Investigators: William L. Pond
Clarence J. Gibbs

Cooperating Units: Pan American Sanitary Bureau
Ministry of Health, Guatemala
University of Miami School of Medicine
Southeast Blood Banks, Inc.
Gorgas Memorial Laboratory, Republic of Panama

Man Years (calendar year 1959):
Total: 2-3/4
Professional: 1-1/4
Other: 1-1/2

Project Description:

Objectives:

Serological studies of sera obtained from Guatemala, Panama, and the Southern United States were planned (and have been accomplished in part) in order to afford guidelines for the interpretation of future serological tests whenever the tests are used as an aid in the diagnoses of diseases of man in the respective geographic areas. Moreover, these serological tests were expected to give an indication, in a rough way, of which arthropod-borne viruses could be present and which viruses are probably absent from the above-mentioned tropical and subtropical areas of the Western Hemisphere.

Methods Employed:

Neutralization, hemagglutination inhibition, and complement fixation tests of arthropod-borne viruses are being carried out on sera obtained by other investigators from residents of Central America and Southern United States.

Major Findings:

The serological test results of sera collected from human beings in eight areas of Guatemala and six areas of the Republic of Panama and the Canal Zone indicate that at least three Group B viral agents may be active in Guatemala and Panama, i.e., St. Louis encephalitis, Ilheus and Yellow Fever viruses. It is realized, however, that in the absence (thus far) of comparable serological tests for dengue inactivity (or other Group B reactivity), one cannot exclude the possibility that the Ilheus and St. Louis encephalitis reactions may be associated with other antibodies of the B Group. The Yellow Fever antibodies cannot, in these tests, be interpreted except as an indication of probable previous infections with Yellow Fever, either from natural infection or infection induced by vaccination.

The serological test results of sera from Miami residents indicate the activity of St. Louis encephalitis, encephalomyocarditis, and possibly even Ilheus virus.

Significance to Program of the Institute:

The potential and actual importance of the tropical arthropod-borne viruses as a cause of infection of residents of Central America and the Southern United States should be determined. This knowledge is a prerequisite to the study of tropical arthropod-borne viral diseases.

Proposed Course of Project:

It is intended that neutralization, hemagglutination inhibition, and complement fixation tests will be carried out on sera not already tested and the serum collections on hand will be augmented with additional sera from the same areas and areas in nearby countries.

Part B included - No

1. [illegible]
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of arthropod-borne viruses in tissue culture. Part 1. Evaluation of tissue culture systems for use in virus isolation, identification, and in serological tests for viral antibodies

Principal Investigator: Charles R. Rosenberger

Other Investigators: Walter L. Newton

Cooperating Units: Laboratory of Germfree Animal Research, NIAID-118

Man Years (calendar year 1959):
Total: 2
Professional: 3/4
Other: 1-1/4

Project Description:

Objectives:

This study is intended to seek out the most sensitive tissue culture cell lines for isolation techniques, to develop a system for virus identification, and to establish suitable tissue culture techniques for serological tests, all in relation to arthropod-borne viruses.

Methods Employed:

A variety of cell types are being tested for sensitivity to arthropod-borne viruses as determined by evidence of cytopathogenic effect, infectivity titers, and degree of propagation of the virus. The decline of activity of the virus under varying conditions without host cells is determined and compared with action of the virus in tissue culture systems. Particular emphasis is placed on determining adequate tissue culture systems for arthropod-borne viruses which are not readily detectable in tissue culture. Several passages of viruses in tissue culture are compared with mouse brain passaged viruses or viruses recently isolated from natural hosts. Various techniques are employed to determine the activity of the viruses in tissue cultures. These techniques include observations for cytopathogenic effect, hemadsorption tests, formation of plaques, microscopic examination of stained cells for inclusion bodies, and serial passages in various tissue cultures and animals.

Major Findings:

Results of the evaluation of the susceptibility of many tissue culture cell lines and the activity of many arthropod-borne viruses using some fifteen different cell lines have shown hamster kidney cell cultures to be outstanding in serving as a culture medium for many viruses. More intensive investigations of a group of nine viruses in hamster kidney tissue culture has shown that on continued passage these viruses lose much of their pathogenicity for mice. A detailed account of this work has been prepared and submitted for formal publication in the near future.

During the course of these investigations and in collaboration with Dr. Walter L. Newton of the Laboratory of Germfree Animal Research, NIAID, kidney tissue cultures from "germfree" mice were prepared and inoculated with various arthropod-borne viruses. Our preliminary results indicate that a greater cytopathogenic effect is produced by dengue Type 1 virus (Mochizuki strain) in germfree mouse kidney cells than in mouse kidney cells prepared from conventional mice.

In investigations of the possibility of undetected viruses in "germfree" animal colonies, no virus could be isolated when tissues were tested in various tissue culture lines and in mice. Certain mouse kidney cell cultures prepared from "germfree" mice have shown some changes suggestive of virus activity. In passages made from these cultures it has been difficult to maintain virus activity beyond three successive passages in tissue culture.

During the course of routine work with hemadsorption techniques in hamster kidney tissue culture a new phenomenon, hemonuclear adsorption, was observed when erythrocytes from one day old chicks were added to hamster kidney tissue cultures which had been inoculated with released viruses. Essentially this reaction results in disintegration of the cytoplasm of the chick erythrocyte leaving the free nuclei which are adsorbed to the infected tissue culture cells.

Significance to the Program of the Institute:

It is hoped that these studies of arthropod-borne viruses in tissue culture will indicate not only the optimal methods for primary viral isolations but also will indicate the best virus-host cell systems for laboratory study of established viral strains.

Proposed Course of the Project:

Studies will be continued along lines which contribute to the basic objectives outlined. Important leads bearing relationship to these projects will be pursued as they arise.

Part B included - No

Serial No. NIAID-9%-B
1. Tropical Virology
2. Arthroped-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part I.

Project Title: Studies of arthroped-borne viruses in tissue culture. Part 2. Development of a tissue culture system utilizing arthroped tissue

Principal Investigator: Charles R. Rosenberger

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1/4
Professional: 1/4
Other: None

Project Description:

Objectives:

To develop a practical tissue culture of arthroped cells which would provide a sensitive and more suitable culture medium for physiological studies of arthroped-borne virus cell-host interactions.

Methods Employed:

Specific tissues are dissected from insects, washed repeatedly in culture media containing antibiotics and placed in culture slides or tubes. The blood of the insect may be collected and added to the culture media. Various culture media and culture techniques are used in attempts to provide a culture system suitable for a particular tissue. Arthropods are occasionally reared under sterile conditions so that the entire animal may be used in culture without contaminating organisms.

Major Findings:

In attempts to culture insect tissue some cells have remained alive for several weeks and in certain instances some apparent growth was observed; however, practical arthroped tissue culture has not been established.

Significance to the Program of the Institute:

Tissue culture of arthropod cells would provide a completely new approach for physiological studies of viruses, host cells, and their interactions. In the field of arthropod-borne virus research, insect tissue culture may provide a sensitive and more suitable culture medium.

Proposed Course of the Project:

Basic studies in the field of tissue culture of arthropods will be continued.

Part B included - No

Serial No. NIAID-95-A
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on antigen-antibody reactions of the arthropod-borne viruses. Part 1. Investigations of the comparative rates of reaction of arthropod-borne viruses with homologous and heterologous neutralizing antibodies

Principal Investigator: Robert M. Pennington

Other Investigators: William L. Pond

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To investigate the arthropod-borne virus neutralization test as a dynamic occurrence. Specifically, the variables of the neutralization test being included in the investigations are time (as an independent variable) and the relative degree of reaction (amount of neutralization). Information from this approach is expected to give insight into the actual kinetics of the antigen-antibody combination and reaction which we usually measure as neutralization of antigen virulence.

Methods Employed:

The rapid *in vitro* combination of antigen with homologous antibody and the comparatively slow speed of reaction (avidity) with heterologous antibody is being successfully applied to markedly increase the specificity of the arthropod-borne virus neutralization tests. Project-type serological tests, results of which are likely to be uninterpretable because of broad cross-reactive antigenic relationships, are being studied by kinetic neutralization studies.

Major Findings:

Neutralizing indices of St. Louis encephalitis antiserum against

St Louis encephalitis virus without incubation and following 2 hour incubations is seen to be 2.6 and 3.0 logs respectively, a difference of only 0.4 of a log. This data seems to indicate that the neutralization of virus by homologous antibody is an instantaneous phenomenon. Moreover, Ilheus antiserum, when tested for neutralization of St. Louis encephalitis virus, had a neutralization index of only 0.6 of a log when tested without prior incubation (0 time) and 1.6 logs if incubated 2 hours prior to incubation into experimental animals. This was interpreted as evidence that the degree of neutralization of virus (antigen) by a closely related heterologous antibody increases as the incubation time is increased. This hypothesis was further strengthened using the JE and WN virus-antisera systems.

Significance to Program of the Institute:

The kinetic neutralization studies present a means for further increasing the specificity of the mouse neutralization test. For the first time in broad serological survey work carried out at Laboratory of Tropical Virology, and other institutions, a means of identifying a specific antigenic stimulus is possible where there once existed a confusing broad antibody profile. Moreover, this study also demonstrates that the basic phenomenon of instantaneous homologous antigen-antibody combination existing in bacterial antibody systems also function in the same manner with virus antibody systems.

Proposed Course of the Project:

The concept of "instantaneous" neutralization of viruses with homologous antibody is now being tested using broadly reactive serum specimens obtained previously from indigenous Southeast Asian patients while in the acute and convalescent phase of some Group B arthropod-borne viral infections. The specific causative agent has not been determined because of the extreme Group B interrelations in sera from residents of this geographic area.

Part B included - No

Serial No. NIAID-93-B
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on antigen-antibody reactions of the arthropod-borne viruses. Part 2. Development of a practical and specific flocculation test for the demonstration of arthropod-borne virus antibodies

Principal Investigator: Robert M. Pennington

Other Investigators: William L. Pond

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/2
Other: None

Project Description:

Objectives:

Development of a rapid flocculation test for the detection of arthropod-borne virus antibodies and its evaluation as a worthwhile laboratory procedure.

Methods Employed:

Arthropod-borne viruses, in the form of infectious tissue culture supernatant fluids, are adsorbed onto clay particles in the manner previously described by Bozicevich of the Laboratory of Immunology for the Bentonite Flocculation Test for Trichinosis and for other diseases. In the same manner also, specific flocculation of the antigen-coated Bentonite particles is demonstrated when in the presence of specific viral antibody.

Major Findings:

The Bentonite Flocculation Test has recently been applied in our Section to the antigen-antisera systems of St. Louis encephalitis, Eastern equine encephalomyelitis, Sindbis, and dengue Type 2 viruses with apparent preliminary success. Satisfactory viral antigens, intended for subsequent adsorption onto clay particles, were prepared from virus suspensions after propagation in hamster kidney

tissue culture systems.

Although the nature of the antibody being measured by this test is, as yet, undetermined, the results of a few tests indicate some correlation between the presence and titer of neutralizing, complement fixing, and hemagglutination inhibiting antibodies and the Bentonite Flocculation Test antibodies. The few observations on the specificity of this reaction indicates that it is as specific, if not more so, than the neutralization test.

Significance to the Program of the Institute:

The Bentonite flocculation techniques, as applied to the arthropod-borne viruses and their antibodies, will constitute another valuable serological test to be used in conjunction with the well-known complement fixation, hemagglutination inhibition, and neutralization tests. Moreover, preliminary observations also indicate that the antigen-Bentonite combination is applicable as a simple rapid method for concentration of virus from suspension and may be of value in subsequent adsorption of selected antibody from serum.

Proposed Course of the Project:

Evaluation of the Bentonite Flocculation Test (as applied to arthropod-borne virus systems) in terms of practicability for use in the field; in terms of specificity, the sensitivity, and the general nature of the antibody being measured; in terms of the interpretation of the results obtained from the test; in terms of its applicability to the diagnosis of disease; in terms of the reproducibility of the results and of the stability of the test reagents; in terms of possible improvements in the methods employed for preparing the test reagents and carrying out the test.

Part B included - No

Serial No. NIAID-96-A
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Transmission of arthropod-borne viruses by mosquitoes. Part 1. Pathogenesis of Ilheus virus in adult and suckling mice

Principal Investigator: Herbert T. Dalmat

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To determine the virus-host relationships of tropical arthropod-borne viruses, using Ilheus fever virus as a model. Determine appropriate experimental host animals for laboratory studies, as shown by viremias developed and infectivity of the animals for arthropods; and by effect of virus dose, route of inoculation, and age of host on viremia and disease.

Methods Employed:

These studies are carried out by means of infecting various species of animals, and also inoculating different aged animals of the same species with varying doses of virus by different routes and determining, by titration of blood in 2½-day-old mice, the amount of circulating virus developing at intervals after infection. Serial passages were made at predetermined intervals in attempts to establish virus strains in a particular animal.

Major Findings:

Chicks are unsuitable as laboratory hosts for Ilheus transmission studies since the proportion of those inoculated that subsequently develop viremia is too variable and those that did develop viremias were non-infective for mosquitoes.

Arthropod transmission of Ilheus virus can be consistently successful if newborn to 8-day-old mice are employed as host animals, and these are best inoculated by the intracerebral route.

Although there is no substantial difference in susceptibility to Ilheus virus in infant and adult mice, there is a marked difference in the development of virus they circulate in their blood.

The amount of virus in the brains of both 3-day-old and 21-day-old mice increases up until 88 hours following inoculation, decreasing thereafter. Since mice usually die 5-6 days after inoculation, perhaps in attempts to obtain maximal amounts of virus routinely infected mouse brains should be harvested 1-2 days prior to the first appearance of signs of disease, as exemplified by a few other viruses such as the Lansing strain of poliomyelitis virus when used to produce infection in mice.

The presence of virus in the lungs of 3-day and 21-day-old mice and the apparent correlation of quantity of virus in the lungs with that in the blood, suggests the possible "spill-over" of virus from the blood to the lungs.

The subcutaneous route of inoculation of Ilheus virus into mice probably is the closest of the usual routes used to that of mosquito transmission by its bite, and results in viremias about equivalent in concentration of virus to that produced after intraperitoneal inoculation, but lower than that following intracerebral inoculation.

Significance to the Program of the Institute:

With our knowledge of the presence of certain arthropod-borne virus diseases in the United States, and the possibilities that others might actually be present or might be introduced from countries to the south, as much as possible must be learned about these diseases, such as which animals might serve as reservoirs, what conditions must be satisfied for transmission to take place, and all other characteristics of the disease. The studies being carried out with Ilheus virus will serve as a model for similar studies of other arthropod-borne viral diseases.

Proposed Course of the Project:

Initiate similar studies with other arthropod-borne viruses. Repeat a limited number of the studies, using a strain of Ilheus which has not been successfully passed numerous times through mice by the intracerebral route, but rather has recently been isolated from mosquitoes in the field.

Part B included - No (Included for this principal investigator in Project Serial No. NIAID-96-B)

Serial No. NIAID-96-B
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Transmission of arthropod-borne viruses by mosquitoes. Part 2. Transmission studies on Ilheus virus using mice as hosts and Aedes aegypti as vectors

Principal Investigator: Herbert T. Dalmat

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To determine the interrelationship of mosquitoes and the tropical arthropod-borne viruses; determine relation of circulating virus concentration in the blood of hosts to infectivity for mosquitoes; determine relationship of duration of virus incubation in the mosquito to the ability of the mosquito to pick up and transmit virus; determine shortest interval after an infectious meal at which a mosquito can transmit virus; where in the insects the virus lodges or multiplies; techniques for better recognizing the presence of virus in mosquitoes.

Methods Employed:

At different intervals after sucking mice were infected, viremias were determined and pools of mosquitoes were permitted to feed on the mice. After appropriate incubation periods, the mosquitoes were tested for their ability to transmit by bite to susceptible mice and also for virus content in their body.

Large numbers of mosquitoes that had previously fed on mice with known viremias were individually refed each day on susceptible mice which were thereafter observed for death and tested for presence of virus.

Major Findings:

Although investigators subsequent to the original isolation of Uhlenbuth virus have been unable to transmit the virus by mosquito bite from animal to animal, we were able to achieve transmission by Aedes aegypti at 7 days after their infectious meal as well as after 12-14 days. Transmission was especially successful when the circulating virus titer in the donor mice was above 6 logs LD_{50}. The number of mice dying after bites by infected mosquitoes was neither an all or none result and was reminiscent of what would be expected if one LD_{50} were to be inoculated, a situation quite difficult to reproduce even with known inocula.

Ninety-eight percent of mosquitoes used in one large study were able to transmit sometime between 2-14 days after their infectious meal while only in 38 percent were we able to demonstrate virus in their bodies by triturating and inoculating body suspensions into mice on days 7-20. Most of the mosquitoes (92%) were able to transmit by bite by day 9 and more were transmitting on days 7-9 than on days previous to or after this interval. In another experiment, the infection rate in the mosquitoes was relatively high throughout, while very few mosquitoes actually transmitted by bite. The viral content of a single infect mosquito appears to increase sometime between 7-10 days after its infectious meal, which corresponds with the period of maximum demonstrable infection rate.

Significance to the Program of the Institute:

Same as for project (Serial No. NIAID-96-A) on "Pathogenesis".

Proposed Course of the Project:

Continue with some of the objectives already stated. Initiate similar studies with other arthropod-borne viruses of the tropics. Determine the most efficient number of mosquitoes to use for consistent results in studies of transmission by bite and in preparing pools for isolation of virus. Determine virulence of virus in dead mosquitoes since in field survey work, mosquitoes are often dead by the time virus isolations are attempted. Repeat a limited number of the studies, using a strain of virus closer to original isolation, to see if the transmission pattern differs from that has been determined with a strain that has already undergone 38 mouse-brain passages.

Part B included - Yes

Serial No. ENTO-96-J

FES-CSS
Individual Project Report
Calendar Year 1959

Part I. Honors, Awards, and Publications

Publications other than abstracts from this project:

Labeyrie, E. A.: Arthropod transmission of rabbit fibromatosis (Shope). J. Hyg. 57: 1-30, 1959.

Labeyrie, E. A. and Scanlon, M. F.: A comparative study of the Shope fibroma in rabbits in relation to transmissibility by mosquitoes. J. Nat. Cancer Inst. 22: 593-615, 1959.

Honors and awards relating to this project:

M. Herbert F. Labeyrie was elected a Trustee of the Thomas Say Foundation, Entomological Society of America (1959 to date).

Serial No. NIAID-9
1. Tropical Virology
2. Arthropod-borne Virus
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Effects of artificially varied environment on potential mosquito vectors of arthropod-borne viruses

Principal Investigator: Paul A. Woke

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

To provide a basis of methods and biological data for planned studies of virus-vector-environment interrelationships, field epidemiological and control investigations of arthropod-borne virus diseases, and of means for maintaining Haemagogus equinus under optimum conditions for use in experimental investigations.

Methods Employed:

Haemagogus equinus, a known mosquito vector of yellow fever, has been studied under varied environmental conditions in the field and laboratory and its responses measured. On the basis of analyses of the results of these studies, methods and equipment have been modified, and new equipment has been devised to improve laboratory culture conditions. Determinations were made of the survivorship distribution under the improved conditions of culture.

Major Findings:

Means were found which greatly improve the culture methods for maintaining Haemagogus equinus. This was accomplished principally by the design, on the basis of biological studies, of new culture equipment which eliminates many of the outstanding conditions that lead to disorders and premature deaths, and which provides more effective conditions for mating, feeding on carbohydrates and on

animals for blood, and for ovipositing, and with greatly increased ease in care and reduced opportunities for escape of the mosquitoes. Environmental conditions were improved by determinations of the more nearly optimum conditions and their provision within the limits of the equipment available. The longevity of caged adults of Haemagogus equinus has been greatly extended by improved methods of culture, females now living up to 94 days, males up to 107 days, in contrast to previous maximum of 69 and 64 days respectively.

Significance to the Program of the Institute:

The results of these studies help to prepare a sound basis for: (1) field studies of survival in the natural habitat and laboratory analytical studies of internal (physiological and pathological) factors in survival; (2) laboratory maintenance of the species under optimum conditions for maximum effectiveness in research, so that (a) results of experimental work may be expected to reflect the potentialities of the species uninfluenced by effects of an inferior environment, (b) the possible selective effects of conditions adverse to the population may be avoided, and (c) standardization of strains for experimental purposes may be accomplished; (3) field epidemiological investigations of arthropod-borne virus diseases; (4) laboratory investigations of virus-vector-environment interrelationships; (5) investigations directed toward the development of control measures against arthropod vectors of virus diseases.

Proposed Course of the Project:

Through studies of the vector in the laboratory and in the natural habitat, using the improved means for maintaining and studying the vector, and with adequate control over the production and management of the conditions known to effect the health and survival of vectors, to determine the approximate survival potential of the species under controlled constant uniform environmental conditions within the optimum range.

Similarly, but in order to more closely approach natural conditions, to study behavior and survival under controlled environmental conditions that are varied through a diurnal cycle approximating usual cycles found in the natural habitat.

To make similar studies, but in which differentials in intensity of different environmental conditions are provided in different parts of the culture chamber.

To apply the findings of these studies and extend the studies to include first generation Haemagogus equinus and to include vector species other than Haemagogus equinus.

To apply the findings to studies in the interrelationships of viruses, vectors, and the environment.

The above proposed studies require equipment which has been requested but is not at present authorized by this Institute for purchase. The equipment currently in use is unsuited for producing scientifically reliable results and lacks essential versatility.

Part B included - No

A REVIEW OF ACTIVITIES OF THE

MIDDLE AMERICA RESEARCH UNIT

April 1958 - September 1959

Middle America Research Unit
Box 2011
Balboa Heights, Canal Zone

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25 September 1959

Introduction

The Middle America Research Unit has been in actual operation for about 17 months at the time of this writing. After the first few months of orientation and staffing, the programs of the two Sections were rapidly developed in accordance with the originally delineated objectives. The Mycology Section has concentrated on histoplasmosis in all of its aspects; the Virology Section has been involved in a dozen specific research projects (half of them completed or nearing completion) with emphasis on the tropical arthropod-borne virus infections, but ranging from EMC infection in swine to human epidemic influenza.

With the cooperation of the Canal Zone and the Republic of Panama medical facilities, including the Gorgas Memorial Laboratory, the position of this laboratory as a research organization has been established. The remarkably effective (inspite of long distances involved) technical and logistic support by the National Institutes of Health and Walter Reed Army Institute of Research makes possible the long range and the day to day operations of the laboratory. Some operational problems remain but these can be resolved with a little patience and effort.

It is safe to predict that if this field laboratory is established on a permanent basis it will eventually develop into a compact but productive tropical research center contributing to our knowledge of diseases important to human and domestic animal health.

I. SUMMARY OF ADMINISTRATIVE ASPECTS

A. Origin of MARU

The Middle America Research Unit (MARU) is an interservice field laboratory sponsored by four of the Uniformed Services of the United States. Currently major operational support is provided by the U.S. Public Health Service through the National Institutes of Health (NIH) with an additional contribution by the Department of the Army through the Medical Research & Development Command (R & D). The laboratory consists of two Sections: Virology and Mycology. The Virology Section of MARU is also a component section of the Laboratory of Tropical Virology of the National Institute of Allergy and Infectious Diseases (the other component of this laboratory is the Arthropod-Borne Virus Section located at NIH in Bethesda). In July 1959 the Mycology Section of MARU acquired organizational autonomicity as the "U.S. Army Medical Research Unit, Panama" (USAMRU). This unit is administratively supported by the Commanding General, U.S. Army Caribbean while the technical supervision has been delegated by the Commanding General, U.S. Army Medical R & D Command to the Director, Walter Reed Army Institute of Research (WRAIR). For obvious practical reasons the Director, WRAIR has further delegated routine and immediate professional supervision of the Army Unit to the Director of MARU.

The establishment of the field laboratory in the Canal Zone was a result of extensive surveillance and negotiations in 1956-1957 between the representatives of the medical research branches of the Uniformed Services with the office of the Secretary of the Army, the Canal Zone government, the Panama Canal Company, and components of the Caribbean Command. A working agreement to serve as a charter for the initiation of activities was formulated by the Assistant Secretary of the Army (FM), the Governor-President of C.Z. & PCCO,, and the Surgeon General, USPHS. This agreement was signed on 16 October 1957. The first paragraph stated in part, "In recognition of the exploratory nature of the proposed research program, it is expected that this agreement shall remain in effect for three years, with a scientific evaluation to be made at the end of the second year concerning the progress to date and the potentialities of further cooperative research in this general location. This interim review will establish the basis either for the discontinuance of this field party or for recommending the renewal of the agreement and the development of a permanent laboratory in the Canal Zone".

The purpose of this report is to summarize some of the information required for such an evaluation.

B. Present Physical Facilities

The summer and fall of 1957 were devoted to planning of the projected laboratory. Upon execution of the formal agreement work began on conversion of Building No. 265 in Ancon, C.Z. located within the Gorgas Hospital complex. The three story building, formerly the nurses' home, was rented for the period covered by the agreement. The poured concrete structure was found to be in good condition except for deterioration of the wood flooring and paneling. The plan of multiple 12 by 12 ft. rooms allowed conversion of the first floor,with minor structural alterations, into a relatively efficient laboratory space. More extensive construction was required to provide modest but adequate glassware and cage washing facilities. A large wing of the first floor immediately adjacent to the laboratory area was reserved for breeding and rearing of laboratory animals. The laboratory proper (2,000 sq. ft.) was air conditioned; the administrative offices, service area, and animal quarters were left without air conditioning. On 27 December 1957 the laboratory was officially established. Because of a delay in construction and other unforeseen complications, the actual laboratory bench work did not commence until mid-April 1958.

The mycological research has been conducted in three laboratory rooms and a separate air conditioned "sterile" transfer room equipped with a hood and ultraviolet disinfection lights. In addition to approximately 580 sq. ft. occupied by the bench area, there is a small screened porch (72 sq. ft.) for maintenance of animals infected with suspected mycotic materials. The virological activities began within an area essentially identical with that described for the mycology group, but of necessity there has been gradual expansion to five rooms for bench work, a room filled with freezers (total air conditioned area of ca. 1,000 sq. ft.) and five rooms for maintenance of infected animals (4 of them on the second floor). A separate room also air conditioned is restricted to preparation of mycological, bacteriological, and virological media. The animal rearing and maintenance have expanded to the second floor area (total area for uninfected animals - 1,500 sq. ft.). Portions of the second and third floors are used for storage of supplies. A basement room is occupied by acid cleaning equipment (stainless steel tanks and cylinders) for special glassware preparation.

The physical facilities are already strained and should the laboratory be continued, immediate conversion of additional space into bench areas is needed to accommodate the present activities. The Virus Section will require prompt air conditioning of a part of the first floor porch to allow removal of the several freezers from a potential extra laboratory room (12 x 12 ft.). Another porch area, reserved for preparation of "normal" cell culture suspensions, their distribution into tubes, and the changing of nutrient media prior to virus inoculation will be set up next to the present media room.

Gradually, the laboratory has become well equipped with all of the essential major equipment and basic services, including the needed number of incubators, refrigerators, -20°C freezers, standard centrifuges, as well as a refrigerated centrifuge with high speed attachment (International PR-2) two Revco freezers (operating at -55°C), and a "Freeze-Dry" lyophilizing machine (Am. Instr. Co.) operated either with CO_2 ice or by electricity.

C. Organizational Structure

Because of the interagency sponsorship and support the organizational structure of MARU is difficult to represent graphically. An attempt to do so is made in Fig. 1. The two sections of MARU are represented in relation to the parent organizations and the supporting home base laboratories. In the case of the Virus Section the immediate assistance is provided as needed by the Arthropod-Borne Virus Section of the Laboratory of Tropical Virology (NIAID) in Bethesda headed by Dr. William Pond. The Mycology Section receives similar support from the Mycology Section of WRAIR headed by Miss Charlotte C. Campbell. It will be noted that the Mycology Section is administratively supported by USARCARIB. The National Naval Medical Research Institute as yet is not contributing to the functioning of the laboratory and is therefore listed merely in the position of a potential parent organization.

D. Cooperating Organizations

Pan American Sanitary Bureau/Pan American Health Organization/WHO

Ministry of Health, Republic of Panama

Ministry of Health, Costa Rica

Gorgas Memorial Laboratory

Department of Health, Colony of British Guiana

Chief Surgeons for the Army, Navy, and Air Force of the Caribbean Command

U.S. Army Mission to Panama

Laboratorio de Diagnostico e Investigacion Veterinaria, R. de P.

U.S. Army Malaria Control and Survey Branch, C. Z.

Preventive Medicine Officer, C. Z.

Veterinary Health Officer, C. Z.

Gorgas and Coco Solo Hospitals, C. Z.

Santo Tomas Hospital, Panama City

Hospital del Nino, Panama City

Universidad del Valle, Cali, Colombia

Sanitary Engineering Division, ICA, Panama City

Military Sea Transport Service

Division of Biologic Standards, NIH

Virus & Rickettsia Section, WRAIR

While difficult to diagram the relationship, both MARU sections maintain a close collaborative and cooperative relationship with the Gorgas Memorial Laboratory in Panama City (Director - Dr. Carl M. Johnson), especially in the activities within the Republic of Panama. In certain of the international projects administrative cooperation is provided by the Zone officers of the Pan American Sanitary Bureau of the Pan American Health Organization (PASB/PAHO).

The Veterinary Laboratory and the U.S. Army Mission to Panama have closely collaborated with both MARU and the Gorgas Memorial Laboratory on several important projects. The U.S. Army Malaria Control and Survey Branch personnel have assisted in the MARU field functions, particularly in the trapping of wild animals and in relieving the entomological load otherwise carried by the GML staff.

E. Research & Supporting Personnel

As of September 1959 MARU is authorized a total strength of 31 positions, of which 28 are provided by the U.S. Public Health Service and 3 by the U.S. Army.

Fig. 2 illustrates the growth of the field laboratory personnel from its inception in December 1957 through September 1959. It can be seen that the personnel of the Supporting Services rapidly increased until January 1959, while the research staff expanded at a still more rapid rate until mid-September 1959.

Director: Dr. Alexis Shelokov

Virology Section
(LTV, NIAID)

Dr. A. Shelokov, Section Head
Dr. Charles G. Dobrovolny
Dr. John E. Craighead
Dr. Jacob A. Brody (EOD 9/17/59)
Dr. Pauline H. Peralta
Vacancy, Med. Officer USPHS
Mr. John E. Vogel
Miss Dorothy Longfellow
Vacancy, Research Techn.
4½ laboratory assistants

Mycology Section
(USAMRU, Panama)

Capt. R. L. Taylor, Section Head & CO USAMRU
Mrs. Martha Shacklette
SP-7 Harlan Kelley
1½ laboratory assistants

Supporting Services
(LTV, NIAID)

Mr. Michael Spontak, Superv. Biol. Tech.
Miss Maria V. Faraudo
Mr. Albert H. Anglin
4 lab. aids
4 animal caretakers
1 messenger-driver
1 night janitor

Present problems in technical staffing of the two sections are quite different. The Mycology Section has been so far concerned primarily with a single disease entity of histoplasmosis, the study of which requires certain specific skills and defined techniques. The small but well trained staff is in a position to perform the mission. On the other hand, the Virus Section was charged with the task of investigating the broad field of virus diseases of public health importancein Middle America. This has required development and use of multiple approaches and skills by a larger technical staff.

F. Dimension of Laboratory Operations

The scope of the expanding laboratory activities can be gaged from a mention of some of the miscellaneous information some of which will not otherwise be covered under the project descriptions.

The laboratory mouse production began in April 1958 with 240 males and 960 females supplied from NIH and WRAIR. Seven months later the production was in full swing with 820 litters (over 6,200 baby mice) born in the 410 mouse cages then maintained. Because of the high cost of imported animal food and problems in maintaining adequate care, the colony has been kept at the minimal required level: during the past 12 months about 55,000 infant mice were born (7,000 litters). With current commitments to a collaborative field project on arthropod-borne viruses with GML the production will have to expand. In addition to the breeding of mice, a number of animals of other species have been continuously housed for regular use (hamsters, guinea pigs, rabbits, chickens, geese, and monkeys).

The Virology Section has logged in its books about 10,000 specimens from many different sources (mosquito pools to rectal swabs) for attempted viral isolations; over 5,000 serum specimens from tropical America (plus 700 "normal" Maryland sera) were entered for testing or storage for future use. Virus isolations have been attempted and made in animals, embryonated eggs, and cell cultures of monkey and hamster kidney, chick embryo, human amnion, HeLa, and other cell types. Cell cultures are observed for CPE and some are subjected to hemadsorption and hemagglutination diagnostic tests. Human and assorted animal sera have been regularly examined for the presence of antibodies inhibiting hemagglutination by the arthropod-borne viruses (especially eastern equine encephalitis) and several myxoviruses. Many of the same sera have also been checked by complement-fixation tests and neutralization procedures were performed in animals or tissue culture systems.

The Mycology Section, besides processing well over 500 clinical specimens and nearly 200 soil samples for isolation procedures, primarily H. capsulatum, performed 850 complement-fixation and 600 collodion-agglutination tests on the suspected serum specimens. In addition, 550 cultures for air borne mold spores have been inoculated.

II. SUMMARY OF RESEARCH PROJECTS, VIROLOGY SECTION

The objective of the Virus Section is to evaluate the significance of viral agents found among the inhabitants of Middle America as related to causation of human and animal disease. Special emphasis is placed on arthropod-borne (ARBOR) viruses, their natural reservoirs and vectors, as well as other agents which may be of potential danger to the populations of other American countries including the United States.

The practical approach to virus field research problems may be divided into long range and short range investigations, both dependent on the cooperation and collaboration with several organizations in the Canal Zone, the Republic of Panama, the health agencies in the neighboring countries, and the support by the Arthropod-Borne Virus Section of LTV in Bethesda (see Section I-D). Long range studies consist of planned investigations to elucidate the significance of finding viruses in the tropical environment by attempted identification of the agents, definition of their natural distribution and reservoirs, possible vectors, and rates of infection as reflected by the frequency of isolation and the presence of specific antibodies in the general population or cases of recognizable illness. Short range studies take immediate advantage of the research opportunities presented by epidemic and epizootic outbreaks and by testing specimens from human and animal cases of suspected viral diseases for the presence of etiologic agents or serum antibody rises. The challenging findings of these emergency explorations often lead to the planned long range projects.

A. Projects in Progress

1. A field study of eastern equine encephalomyelitis in Panama. In August 1958 this laboratory assisted Maj. T. Murnane (U.S. Army Mission to Panama assigned to the Laboratorio Veterinario) in identification of a viral isolate from a suspected equine case of encephalitis histologically confirmed as viral encephalitis at GML. After the second isolation of what proved to be the EEE virus, the Ministry of Health, R. de P., requested the Director of GML to investigate the occurrence of the fatal horse cases as a possible threat to human and animal health. GML established a field station near the affected corral in Pacora (40 miles from Panama City) which was operated for two months with the assistance of MARU. The purpose of the field collections was to attempt demonstration of the local vector of EEE virus (in the absence of Culiseta melanura usually implicated in the Eastern U.S.) and its natural reservoir. During the initial field study the GML field staff selectively collected and identified more than 25,000 mosquitoes belonging to at least 11 different genera. The frozen pools were subsequently tested for virus isolation in suckling mice and tissue culture in the two laboratories. With the advent of the dry season the collections were suspended, but as the mosquito population began to increase in April 1959, the study was resumed with the assistance of the U.S. Army Malaria Control Team (under the direction of Maj. R. Altman and Mr. M. Keenan) because the GML staff was temporarily occupied with other commitments.

While this new collection was in progress in August 1959 two horses dying of clinical encephalitis were brought to Maj. Murnane's attention occurring near the Tocumen airport, R. P. and Curundu, C. Z. respectively. The brains were repeatedly examined for viral isolation with negative results, but histological examination of both brains at GML revealed extensive encephalitis consistent with viral infection. The other common

causes of encephalitis in this area were reasonably ruled out. The terminal "acute" blood specimens from both animals did not contain demonstrable antibodies against EEE virus by neutralization or complement-fixation tests, but both had high hemagglutination-inhibition titers (1:20,000 and 1:40,000). The Pacora site was temporarily abandoned; the nearby Tocumen airport area was covered by the GML staff, while the Malaria Team collectors shifted their activities to the Canal Zone.

Table 1 summarizes the mosquito pool work at MARU during the 1958 and 1959 studies. The virus of EEE has not been isolated from any of the pools, inspite of inoculation into suckling mice (at MARU and GML), and the tissue culture systems of hamster kidney and chick embryo. From several mosquito pools, particularly Culex dunni other ARBOR viruses were isolated at GML and MARU; their nature as yet has not been fully clarified.

Table 2 summarizes preliminary studies on the sera collected from various sources in or near Pacora by MARU and Lab. Vet. staff in an attempt to establish the possible natural reservoir of EEE in Panama. A major effort was devoted to a study of birds. In October and November 1958, 210 wild birds representing 55 species of 29 families were taken by shooting or trapping by MARU field investigators working jointly with the GML staff (unfortunately, this represented only a small sample of the avifauna in the area as indicated by the bird checklists with over 80 families and 1,400 species known to occur in Panama). All of the avian serum specimens were checked for the presence of EEE virus in chick embryo cell cultures. Practically all of the sera were tested for the presence of HI antibodies after acetone extraction by the method of Casals and Clarke (Table 3). Sera from six birds inhibited hemagglutination at a dilution of 1:80 or greater and were considered "positive": 2 boat-bill herons, 1 blue heron, 1 meadow lark, 1 flycatcher, and 1 toucan.

Of the 97 horse sera obtained in the Pacora area, prior to the mass vaccination by the Ministry, 41 were found to contain neutralizing antibodies against EEE virus. Eighty four of these horses were pastured near the tidal lowlands and 38 of them (45%) contained neutralizing antibodies, while only 1 of 13 horses kept in the stables had antibodies (8%). The difference between the two groups is suggestive of the pasture exposure and points to the mosquito vector with short flight range. Over 100 horses from other areas of Panama have been compared with the base line studies at Pacora with varying incidence of positivity possibly related to similar ecological factors.

Serological studies were performed on 222 sera from residents of 13 villages in and near the Pacora site. Only 3 sera were found positive by neutralization and hemagglutination-inhibition tests. If the population is divided into groups of residents in the savanna terrain (102), transitional terrain (74), and tidal lowlands (46), the percentages of serum positivity are 0.0%, 1.5%, and 3.5% respectively. Several groups of human sera from other representative areas of Panama are under comparative study.

In February 1959 exploratory complement-fixation tests were initiated and with the procurement of commercial (Lederle) antigens for EEE and WEE, a procedure utilizing 50% endpoint was found to give satisfactory reproducible results. Over 500 CF tests have been run on 187 horse and 209 human sera, primarily from the Pacora study area. Forty seven of the horse sera were reactive with the EE antigen. The majority of the positive reactors (17/47) was from the horses bled at the time of the outbreak in September 1958. While a single serum reacted with WE antigen alone, 13 additional horse sera were almost equally reactive with the EE and WE antigens, suggesting that another group A virus is active in the area. The pattern is different in case of the human sera: 192/209 were negative with both antigens, a single serum was reactive with EE, and 2 sera with WE antigens; 13 sera were reactive with both EE and WE antigens providing further evidence of possibly another group A virus.

The problem of the comparative significance of the antibody titers as demonstrated by the neutralization, HI, and CF tests in relation to previous recent or distant infection with the EE virus became a major concern of the MARU staff. Some of the comparative data are shown in Table 4.

In the course of TC studies with the EEE virus, the Panama strain was adapted to monkey kidney tissue culture with characteristic and recognizable CPE. Were rhesus MKTC easily available in this laboratory, this could constitute a practicable testing system (particularly because hemadsorption with guinea pig erythrocytes is demonstrable at pH 6.2 even in the absence of CPE).

In summary, the Pacora EEE studies developed into a major MARU project, even though the initial questions posed before the collaborative group were answered only in part. The joint studies of GML, Lab. Vet., and MARU showed that the EE virus was not spreading among the horse or human populations to endanger the suburban communities adjoining Panama City and the Canal Zone. It is likely that through the years the EE virus has been active on the Isthmus of Panama possibly together with other group A agents. It is suspected that the vector is a Culex mosquito. The shore birds appear to be infected more frequently than the inland birds; the actual reservoir of the EE virus has not been established. The unsolved problems undoubtedly will be further clarified during the GML-MARU project in Bocas del Toro (see Section V-A) and continuing surveillance in other parts of Panama.

2. Serological evidence of other ARBOR viruses in Panama. The extensive collection of human and animal sera from a variety of ecological environment procured as part of the EEE study is being tested for the presence of antibodies for the two of the group B viruses, Ilheus and SLE, known to be active in Central America (see Tables 2 & 3). These sera will be utilized for further exploratory serological surveys at MARU and with locally undesirable "foreign" viruses at the Bethesda laboratory.

3. Enterovirus flora of Panamanian children, 1958 & 1959. Many Central American clinicians state that among their more seriour problems are the cases of children with diarrhea and severe respiratory infection. With the increased use of TC technique information on viral causation of these clinical syndromes is rapidly accumulating in North America and Europe, but relatively little is being done in Central and South America.

As a pilot study, and without attempting to explore the possible bacterial etiology of the cases, pediatric ambulatory patients with diarrhea, with severe respiratory symptoms, and with undifferentiated febrile illnesses (FUO) were sampled (by a MARU staff member) by collecting both rectal and throat swabs. This was continued for a period of six weeks in the middle of the "wet season" of 1958. The throat swabs were tested in both MKTC and human amnion while the rectal specimens were inoculated into MKTC alone. All tubes were observed for development of CPE and the throat swab cultures were also tested for hemadsorption with guinea pig erythrocytes.

A total of 231 infants were thus examined with 455 swabs (excluding the contaminated) subjected to actual testing. From 41 of the children 52 viral strains were isolated: the throat swabs yielded 24 and rectal swabs 28 strains. The following table summarizes the 1958 virus isolations by their origin.

TABLE 5

RESULTS OF A 6 WEEK SURVEY OF 231 CHILDREN IN 1958

Virus Group	Throat Swabs	Rectal Swabs	Total
Adenovirus	6	0	6
Myxovirus	3	0	3
Poliovirus	4	1	5
ECHO	2	8	10
Coxsackie B (or Ag)	3	7	10
Unidentified	6	12	18
Total	24	28	52

A longitudinal study of at least one year's duration was initiated in May 1959. Rectal swab specimens alone are collected by a MARU medical officer on all ambulatory clinic infants (rather than children) at the Hospital del Nino once every two weeks. Usually 30 to 40 children are sampled on each occasion. It is too early to discuss the results of this study. Table 6 summarizes the isolations for the collections during May, June, and July 1959. Seventy additional specimens were collected in August but the results are are as yet incomplete. Generally a morning's collection contains about 30-35% specimens positive for enteroviruses. It appears that the isolation rate drops off with increasing age and that the infants under two years of age constitute the optimal source for survey purposes. (The lower percentage of positive isolations during 1958 may have resulted from less optimal storage conditions and sampling of older children.) Identification of the many isolates has begun.

It is anticipated that the exploratory study in 1958 and the longitudinal study of 1959-60 will serve as a baseline for future studies of enteroviruses in Panama, particularly as related to the projected studies on the viral etiology of diarrhea.

4. Ft. Kobbe outbreaks.

a. Epidemic "RUQ syndrome" outbreak. The U.S. Army dispensary at Ft. Kobbe reported in July 1959 a dozen cases of fever invariably associated with RUQ pain and tenderness and monocytosis as a component part of the clinical picture. From one of the cases, 2 virus strains were isolated in MKTC and in 1 day old infant mice (the latter strain has been adapted to MKTC). This patient had homologous antibody rise (>8x) against the MKTC isolate (the SM isolate is being tested). The illness of the other patients from whom paired sera were available is being related to the virus isolates.

b. The "jungle fever" outbreak. In late August 1959, 5 cases of fever and ? signs of meningeal irritation appeared among 200 U.S. soldiers within a week upon return from 4 weeks of training in jungle warfare. The hospitalized patient and 4 ambulatory cases were examined; 51 other soldiers from this company were bled to be followed by appropriately timed second serum specimens. The acute materials were inoculated into SM and TC systems, while the sera will be intensively investigated for possible relation to the ARBOR viruses known to be prevalent in this area. The studies for viruses not known to be active in Panama will be done at the Section of ARBOR Viruses of LTV in Bethesda.

5. Etiology of exanthem subitum. The unusual medical care arrangements in the Canal Zone offer the opportunity of seeing more cases of such minor illnesses as the exanthem subitum (roseola infantum). Dr. D. Hirschl, Chief of Pediatrics, Gorgas Hospital, has initiated a collaborative study to attempt isolation of the etiologic agent of this exanthem.

6. Miscellaneous clinical specimens. It is obviously essential to offer a certain amount of viral diagnostic service to the C. Z. and Panamanian hospitals and physicians. During the past 16 months of laboratory operation, over 2,000 individual specimens were logged under this category representing approximately 700 patients with various and sundry symptoms. (Specimens submitted under specific projects are not included in this category.) In the last 6 months (March-September 1959) 500 miscellaneous clinical specimens were inoculated into MKTC; 50 into special TC systems and 300 into 1 day old SM. Because of the relative unproductivity of attempted isolations from these specimens from sporadic cases (some enteroviruses, myxoviruses, and few SM agents) the Section is contacting the medical profession to establish more rigid criteria for specimen acceptance with preference, of course, for community or familial outbreaks of febrile illnesses.

B. Projects Completed or Near Completion

1. Fatal disease of swine due to encephalomyocarditis virus. The outbreak on a large commercial swine farm located 10 miles west of Panama City, R. P. was investigated in July 1958 by Maj. T. Murnane of Lab. Vet. Approximately 30 pigs died over a 20 day period. Eight of these pigs were autopsied. The outstanding gross and microscopic lesion was the severe myocarditis. The involvement of the CNS was minimal, (Dr. H. Mondragon, Gorgas Hospital).

An agent pathogenic for mice was repeatedly isolated from the autopsy materials and was identified by neutralization test in mice and TC as the EMC virus. Neutralizing antibodies were found in a pig with febrile illness and in the sera of rats (Rattus rattus) trapped on the farm in April 1959. The disease was successfully produced by inoculation of 2 young pigs with the mouse passaged virus and the outstanding pathological finding was the myocarditis. In the course of these studies the EMC virus has been reisolated and passaged in cell cultures of rhesus monkey kidney, hamster kidney, and HeLa. The HeLa cell system has become the standard method of determining antibody levels in experimental and survey sera. An agglutinin for sheep erythrocytes has also been demonstrated in infected mouse brain tissue and in hamster kidney culture fluid.

To our knowledge this was the first demonstration of natural infection of swine with the EMC virus and the most extensive outbreak of EMC infection in men or animals in which the virus was recovered. Contamination of food and water with the excreta of infected rodents and swine were suspected of contributing to the spread of the virus in this outbreak and these factors are under investigation at present.

Because of the possible significance of these findings, studies are in progress at present for investigation of the role of EMC in causation of human and animal disease in Panama. Abattoir swine sera will be tested; rats are being collected from the sewers of Panama City for viral isolations and antibody determinations; paired sera from obscure cases of human FUO, especially with cardiac or CNS involvement, will be tested; paired sera from human cases of myocarditis (kindly offered by Dr. C. Johnson of GML from his Chagas disease serum collection) will be checked; sera from indigenous Indian populations living under primitive conditions will be surveyed.

2. Epidemic influenza in Middle America.

a. Asian influenza in Panama, 1957. The earliest laboratory procedure performed in relation to MARU's mission was an examination of 50 paired sera submitted by GML from patients seen at Gorgas Hospital Clinics with suspected Asian influenza during late 1957. In January 1958 the sera were tested by the MARU staff. Of the 50, 27 had fourfold or higher rises in specific antibodies, and 23 showed no significant antibody rise.

b. Influenza B in Panama, 1959. In June-July 1959 an epidemic of clinical influenza swept the Isthmus of Panama. It began on the Atlantic side where it was recognized in pediatric patients by Dr. C. Abildgaard of Coco Solo Hospital. With isolations of influenza virus in MKTC, detected both by CPE and hemadsorption reaction, and subsequent identification in this laboratory as influenza B (similar to or identical with the strains B/Md/59 and B/GL/54) the surveillance on the Pacific side (Gorgas OPC) by Drs. D. Ford and A. Blanshaft resulted in detection of many early acute cases.

Throat swabs from 59 patients were tested and 23 isolates were typed as influenza B; 39 paired sera from the same patients were tested with 27 exhibiting homologous diagnostic rises (none with rise vs. influenza A_2); 12 of the serum pairs were from patients with positive virus isolation - all 12 exhibited a diagnostic rise in influenza B antibodies. (A single untypable hemadsorption virus was isolated from a suspected clinical case and this patient had no rise in antibodies against either influenza B or A_2).

c. Asian influenza in British Guiana, 1959. In June 1959 there also occurred an extensive outbreak of epidemic influenza in the Colony of British Guiana. By arrangements with the colonial government and the U.K. Embassy in Washington, MARU was directed to assist in the virological studies. Dr. J. Craighead spent 24 hours in Georgetown, B.G. where,with the cooperation and assistance of the Department of Health staff, he collected 50 isolation specimens (21 throat washings, 28 throat swabs, and 1 specimen of lung) and 34 acute phase patient sera. Some of these specimens had been previously preserved under suboptimal conditions which may account for the isolation of only two strains of influenza virus in MKTC by the hemadsorption reaction (without apparent CPE). The strains were similar to influenza strain A_2/Asian/Jap/305/57. In addition, an adenovirus strain and an enterovirus were isolated from these materials.

Collection of the convalescent sera presented a problem: so far 20 pairs have been received and tested; 6 showed ≥4x rise in antibodies against A_2 antigen (none vs. influenza B). The unlikely epidemic role of the adeno- and enterovirus strains will be checked serologically (re Australian experience in 1959 with mixed virus epidemic).

3. Virological surveillance during live poliovirus vaccination program, Costa Rica. In May 1959 MARU was instructed to support the Costa Rica national program of vaccination with live poliovirus vaccine (Lederle) conducted by the Ministry of Health under the sponsorship of the PASB/PAHO/WHO. A group representing the agencies involved travelled to San Jose, Costa Rica to initiate the surveillance program. Arrangements were made for throat, fecal, spinal fluid, and serum specimens from suspected cases of CNS disease to be shipped for viral isolation and serological studies to MARU and to the collaborating laboratory in Cali, Colombia (operated under the sponsorship of PASB within the laboratories of the medical school of Universidad del Valle - Chief of TC Laboratory, Dr. Hanna Doany).

A total of 186 specimens from suspected CNS disease cases was received between May and August, covering the first phase of the program (administration of the polio vaccine of each one of the three types separately within the limits of Metropolitan San Jose area). All the isolation specimens, including sera from the acute cases, were inoculated into 2 groups of MKTC tubes (with and without polyvalent poliovirus antiserum) as well as into suckling mice of less than 1 day of age. The notable features have been the absence of clinical poliomyelitis among the vaccinated children (ages 6-10) and the scarcity of poliovirus isolates in the materials submitted from the suspected cases. To date the isolations were: from one of the unvaccinated paralytic cases a poliovirus type 2; from an afebrile child with questionable and transient weakness of one leg, poliovirus type 1 and Coxsackie B_4 (the onset of symptoms was 4 days prior to the administration of type 1 vaccine which was undoubtedly responsible for the presence of this virus in the stool specimen collected the day after); a type 3 poliovirus from a young infant who did not receive the type 3 vaccine and resided in the unvaccinated area (his symptoms were those of encephalomyelitis rather than classical paralytic poliomyelitis); an ECHO 8 virus from a 2 year old child without systemic symptoms, but a persistent slight limp.

Thus, the virus isolation studies have been singularly unimpressive. All available paired sera from patients and known contacts will be subjected to a determination of the possible diagnostic rises in antibodies against the three types of poliovirus by the metabolic inhibition test (to be done at DBS, NIH). Isolates will be related to the patient's illness by the usual serologic procedures. The poliovirus strains isolated, particularly from non-vaccinated individuals with clinical illness in possible contact with the vaccinees, should be thoroughly investigated as being related or unrelated to the live poliovirus vaccine strains by employment of the several available virus markers.

4. Viral CNS disease in a Guatemala nursery. Since late 1958 Dr. E. Rodriguez Rouanet of the Polio Clinic in Guatemala has been submitting specimens on suspected cases of poliomyelitis, including tissues obtained at autopsy. Poliovirus type 1 was repeatedly isolated from these specimens.

An infant nursery in Guatemala City had no suspected cases until late June 1959 when two patients developed paralytic poliomyelitis resulting in death of one. In the weeks following 14 additional cases of transiently paralytic or non-paralytic disease clinically diagnosed as poliomyelitis (positive spinal fluid findings and the occurrence of the initial paralytic cases). Because of the recurrence of the cases in the end of July, Dr. Shelokov spent 2 days in Guatemala on a consultation visit with the Ministry of Health collecting epidemiologic information and additional materials for virus isolations from the nursery infants, who had remained well, and the attending personnel. So far 14 enterovirus strains have been isolated from the Hogar Cuna rectal swabs and in one case from the CNS tissue of a fatal case. While the latter was type 1 polio, and 2 isolates from the well infants were polio type 3, the remainder are still under investigation: the clinical cases of aseptic meningitis and several other asymptomatic were harboring ECHO rather than polioviruses (so far ECHO 2 and ECHO 5 have been incriminated).

5. Polio type 2 epidemic in Nicaragua. In August 1958 10 of the 12 rectal swabs procured from Managua paralytic cases through the courtesy of the Surgeon's Office, USARCARIB produced typical polio-like CPE in MKTC. Three representative strains were typed at MARU as poliovirus type 2. Comparison of these results with Dr. John R. Paul's data on 35 Managua patients disclosed that 7 of the same patients' specimens were also included in the Yale sampling and were found to contain polio 2 virus. These findings were important because Nicaragua outbreak was the first epidemic of any size attributable to type 2 polio.

6. Concurrent encephalitis in horses and pheasants in Panama. In April 1959 Dr. Carl Johnson of GML alerted Lab. Vet. and MARU of the occurrence of clinical encephalitis among horses on a ranch in the suburbs of Panama City where the EEE virus was thought to be active 3 years ago. At the same time, Maj. T. Murnane of Lab. Vet. began investigations of encephalitis at the only pheasant farm in Panama, only 1 mile away from the ranch where the horses were dying and near still another site of previously suspected equine encephalitis activity.

Several of the horses were ill and two died. The clinical history was typical of equine encephalomyelitis; histological examination of the brain by Dr. Johnson at GML revealed findings "consistent with viral encephalitis or trypanosomiasis". Isolation attempts in two of the laboratories from the brains of the dead horses were negative. In the meantime, the birds at the pheasant farm continued to die from clinical encephalitis.

Specimens from two of the pheasants were examined at Lab. Vet. and from one at MARU: a neurotropic agent from the brain at Lab. Vet. and from the spleen and liver at MARU were isolated in SM. Suckling mouse brain material produced no CPE on inoculation of MKTC, but addition of guinea pig erythrocytes showed definite hemadsorption. This, coupled with the death of chick embryos on inoculation with the pheasants material and Maj. Murnane's clinical impression, made the Newcastle disease virus the prime suspect. A hyperimmune NDV serum was obtained from NIH and tests on the hemagglutinating TC fluids and the allantoic fluids demonstrated that the virus indeed was that of NDV rather than EEE.

Serum specimens from the encephalitic and clinically unaffected horses demonstrated absence of antibody of EEE in most animals and unchanging levels in 3, the latter without relation to the occurrence of clinical symptoms. The sera from 10 of the horses were shipped to the U.S. Department of Agriculture Laboratory at Beltsville, Maryland for CF tests for trypanosomiasis. Two of the 10 sera (both from fatal cases) gave a 4+ reaction with T. equiperdum antigen, making the diagnosis of encephalitis due to T. hippicum almost a certainty.

7. CA virus infection in adults, Panama. In April, May, and June 1958, during a prolonged outbreak of respiratory illness among residents of Panama City, 3 strains of a non-cytopathogenic hemadsorption virus were isolated in human amnion cells. The isolations could not be made in MKTC system and only with continued passage the agent was adapted to HeLa cells. It still does not produce CPE, but can be detected by hemadsorption. Careful studies demonstrated that this virus was identical or closely related to the CA virus of Chanock (Myxovirus parainfluenzae 2). This is of interest because the Panamanian strains were isolated from adults with systemic illness, rather than from children, and without association with the croup syndrome. Successful adaptation of the strain to HeLa cells has facilitated further epidemiologic studies on this virus in the American tropics.

III. SUMMARY OF RESEARCH PROJECTS, MYCOLOGY SECTION, MARU

The Mycology Section of MARU was established by Miss Charlotte Campbell who was in Panama from April until July 1958. The areas of investigation for which the Section was established and the objectives of the Section are as follows:

1. To determine the extent of histoplasmosis on the Isthmus of Panama and the significance of this disease in military personnel stationed in an area of histoplasmosis endemicity.
2. To explore the epidemiology and ecology of Histoplasma capsulatum on the Isthmus of Panama and Central America.
3. To provide diagnostic aids and adequate clinical follow up on all recognized cases of histoplasmosis in this area.
4. To evaluate experimental therapy of histoplasmosis (in cooperation with Gorgas Hospital).

The facilities available at MARU enable the Mycology Section to perform both isolation and serological procedures for mycotic diseases.

Isolation procedures for mycotic agents from clinical materials and sources in nature. Bacteriological media are available for sterility testing and limited diagnostic work. A 20x8 ft. animal room, well isolated from the stock animals, enables the performance of large experiments utilizing mice available in sufficient quantities. This animal room has been utilized for housing larger animals including spiny rats, bats, opossums, guinea pigs, and monkeys.

The Section routinely conducts the 50% endpoint complement-fixation test and collodion-agglutination test for histoplasmosis. The complement-fixation test is utilized as a diagnostic aid for histoplasmosis and both the yeast-phase and histoplasmin antigens are routinely employed, while histoplasmin is the only sensitizing antigen used in the collodion agglutination tests.

The Mycology Section of MARU is dependent upon the Mycology Section of WRAIR for support in the following areas: 1) antigens for complement-fixation and collodion-agglutination tests; 2) sheep cells for complement-fixation tests; 3) liason for procurement of histoplasmin for skin test surveys and other problems that require personal attendance in the United States.

The Section's investigations of the many aspects of histoplasmosis as it occurs in Panama are summarized and evaluated under the headings of histoplasmin skin testing program, ecological studies, and human clinical disease.

A. Histoplasmin Surveys of Human Population

The principal tool throughout the world for the determination of previous exposure to H. capsulatum is the histoplasmin skin test. This has been a basic procedure in the study of histoplasmosis in Central America.

1. MSTS arrivals. The original project established at MARU was the skin testing of military personnel arriving in the Canal Zone for duty. The objectives of the project are to determine the number of non-hypersensitive individuals to both histoplasmin and tuberculin immediately upon arrival in Panama and to retest all negative reactors at 6 to 9 month intervals during their 3 year tour in Panama. This approach should give an indication of the percentage and rate of histoplasmin sensitivity conversion in military personnel stationed in an endemic area. The epidemiological and clinical follow up of all histoplasmin converters will also furnish information regarding morbidity and possible leads to the source of infection.

Approximately 1,025 military personnel and dependents were tested from April 1958 to July 1959 upon arrival in the C. Z. aboard MSTS ships. The primary testing was discontinued as of July 1959 and the retesting of the negative reactors was assumed by the USARCARIB Surgeon's Office.

Continuation of the 6 month retests, until July 1960, should yield information on 700 individuals who will have been retested at least twice (many will have been retested three and four times). The importance of the disease in a military population required to operate in a highly endemic area can best be evaluated at the conclusion of the retest period. The data currently available indicate an approximate conversion rate of 20-25 percent during the first 6 months in the area. It is noteworthy that the vast majority of hospitalized clinical cases of histoplasmosis in the C. Z. have been military personnel and dependents. Future plans are for the USARCARIB Surgeon's Office to continue the retesting program until July 1960 and to reevaluate the project at that time.

2. Canal Zone school system survey. A tuberculin and histoplasmin skin testing program in the Canal Zone school system is being conducted as a joint effort of MARU and the Canal Zone Health Department. The project was originated by Dr. E. Osterberg, Prev. Med. Officer, C. Z. and MARU is utilizing the opportunity to apply histoplasmin skin tests simultaneously with the tuberculin survey.

The project was initiated in March 1959. The Balboa Junior College, High and Junior High Schools, were completed in March. The children in the C. Z. Latin elementary schools should be completed by December. Response to the program has been good; a rough tabulation of the results so far available is as follows:

TABLE 7

U.S. RATE SCHOOLS, C. Z.

Pacific Side		Atlantic Side	
Grades 7-12		Grades 7-12	
No. of Tests	% positive	No. of tests	% positive
1606	60%	447	78%

LATIN AMERICAN SCHOOLS, C. Z.

Pacific Side		Atlantic Side	
Grades 1-6		Grades 1-6	
No. of tests	% positive	No. of tests	% positive
863	66%	534	27%
Grades 7-12		Grades 7-12	
No. of tests	% positive	No. of tests	% positive
769	83%	704	44%

It is anticipated that approximately 3,500 histoplasmin skin tests will be completed in the U.S. elementary schools before December 1959. The available data, analyzed on the basis of duration of residence on the Isthmus, show a step wise increase in hypersensitivity. Analysis of the final data will be made to include: size of reaction, sex, age, race, and duration and location of residence on the Isthmus.

Future plans include the retesting of a small group of school children at 3 month intervals and/or the testing of an entire residential community to obtain the percentage of histoplasmin reactors from 1 through 60 years of age.

3. Pre-school children survey. In an effort to cover the younger age groups Dr. C. F. Abildgaard, Chief of Pediatrics, Coco Solo Hospital, has been histoplasmin-testing the pre-school age children seen in the out patient department. His plans are to continue the program for one year at which time he hopes to have data on 1,000 young children.

The preliminary 6 month retesting of previously negative children has been initiated. The data to date show:

TABLE 8

Age	No. Tested	% positive
0-12 months	45	2
1-2 years	141	6
3-4 years	72	9
5-6 years	60	11
Total	318	7.5%

4. Additional histoplasmin testing. A histoplasmin survey was conducted during February 1959 in Guatemala, C.A. where 1,094 persons were skin tested. Ten areas were selected for testing in an effort to obtain information from the differing climatic and ecological environments. The overall sensitivity was 58.5%. Analysis of the results indicates that 63.7% of the male population is sensitive as compared to the 49.3% positivity for the female group. Further separation of the data according to climatic and topographical areas showed the highest percentage of reactors to be 80.7% in the lowlands covered with tropical rain forest. The lowest number of histoplasmin reactors was found in the high, semi-arid region where only 27.7% of the people were found to be sensitive. The results of this survey furnished the only information on histoplasmin sensitivity in Guatemala and may provide an insight into the tropical ecological habitat most suitable for distribution of H. capsulatum.

Primarily as a by product of our other histoplasmin skin test programs, data are accumulating on hypersensitivity in long term adult residents of Panama and the Canal Zone. To date the data on U.S. citizens with over 4 years residence indicate histoplasmin sensitivity approaching 90%.

B. Ecological and Epidemiological Studies

The attempts to determine the enzootic and ecological habitat of H. capsulatum in Panama, as related to human epidemiological pattern, have employed isolation attempts from soil and animal tissues and animal skin testing.

1. Experimental infection in animals. The spiny rat was chosen (Mr. M. Keenan, U.S. Army Malaria Survey Branch, reports that it comprises 80% of the ground mammals trapped in Panama). Experimental infection of spiny rats with a subsequent demonstration of a rise in collodion agglutinins and complement-fixing antibodies in addition to recovery of H. capsulatum, preceeded their routine trapping. These studies resulted in the demonstration of histoplasmin hypersensitivity and the development of a satisfactory skin testing technique.

The skin testing of spiny rats was accomplished only after resolving some unexpected difficulties (the loose body skin tears easily) by devising a technique utilizing the rat's ear. The skin tests are conducted using 1:10 histoplasmin and are read at 24 hours which was determined as the optimal time after observing the tests at 24, 48, and 72 hours.

The maintenance and infection of fruit and insect bats was undertaken with limited success. Fruit bats were inoculated intraperitoneally and the organism recovered 2 months later. Extreme difficulty was encountered in attempting to keep 10 to 20 bats alive for any period of time. Antibodies were not demonstrable in the small quantities of bat serum obtained since every serum was anticomplementary in the complement-fixation test.

2. Puma Island. The site in Panama that has received the most attention has been the Puma Island located on the Atlantic side of the Isthmus in Gatun Lake. The reason for concentrating on this spot was the limited size of the Island and the fact that it had previously been inhabited for a 2 year period from 1955 to November 1957 by a small number of U.S. Army personnel 10 of whom developed clinical histoplasmosis during their stay on the Island.

Forty three soil samples collected on Puma Island, both at the sites of former living quarters and from animal burroughs, have failed to yield H. capsulatum. The U.S. Army Malaria Survey Branch offered the services of a group of men to trap animals on Puma and from October 1958 to March 1959 the Island was systematically covered, although the only animals trapped were spiny rats and opossums.

The animals were transported to the Pacific side, where the Mycology Section personnel bled and skin tested them with 1:10 histoplasmin. The animals were maintained at MARU for approximately one week until the completion of the collodion-agglutination and complement-fixation tests.

The skin test positive animals with negative collodion and complement-fixation reactions were sacrificed and liver and spleen inoculated into mice and onto nutrient media for recovery of H. capsulatum. All animals having collodion-agglutinins or complement-fixing antibodies were rebled and titers followed for periods up to two months. The animals were then sacrificed for recovery of H. capsulatum. The results of the animal work are listed below.

TABLE 9

ANIMAL COLLECTION ON PUMA ISLAND

	Opossums	Spiny Rats	Bats	Golden Anteater
a) Total collected	28	161	31	1
b) No. ST+ (1:100) *				0/1
c) No. ST+ (1:10)	4/27 **	66/161	9/31	
d) No. (+) sera	0/14	32/105	0/8	
e) No. (+) by culture	2/17	5/90	0/12	

* ST = skin tested.

** Numerator = No. pos; denominator = number tested.

The animal trapping experiments proved that the organism H. capsulatum is still on Puma Island which has been uninhabited for 2 years and that animals living on the island are naturally infected with H. capsulatum. The isolation of H. capsulatum from wild animals had not been accomplished in Panama before and these results may provide information on the longevity of the organism in an isolated and uninhabited area.

3. Histoplasmin survey in dogs. A reported occurrence of histoplasmosis in dogs (1951) lead to a skin test survey of dogs at the animal clinic, as well as dogs under the compulsory 4 month quarantine. This survey has been conducted by Dr. R. B. Wallace of the Corozal, C. Z. clinic. Dr. Wallace skin tested more than 150 dogs and has yet to find one positive reactor. He has discontinued routine testing and uses histoplasmin only as a diagnostic tool. There are no plans to continue this survey.

4. Soil isolation studies. In addition to the soil samples collected on Puma Island, specimens have been collected from the Canal Zone as well as the Republic of Panama for isolation of H. capsulatum. The tabulation of the results from the soil collections is as follows.

TABLE 10

SOIL COLLECTIONS

	Puma	Madden Preserve	Other *
a) Total collections	39	14 **	23
b) No. pos. H. capsulatum	0	7	0

* Collected at homes of patients with clinical histoplasmosis.
** 3 positives are from same site collected during 1) dry season, 2) at end of rainy season, and 3) during next rainy season.

Results are not yet available on the 45 soil samples recently collected in conjunction with clinical cases of histoplasmosis and the 8 soil samples collected from tree buttresses on Puma Island.

The percentage of current isolations is remarkable in view of the earlier unsuccessful attempts by Miss Campbell and Dr. Emmons who processed many soil samples. Only one previous isolation has been made from 100 Panamanian soil samples processed by Dr. L. Ajello of CDC. (All of the recent isolations were made from soil collected by Mr. C. Hansen of GML from the same type of ecological environment and specifically selected for the probable recovery of phlebotomus larvae; the samples were collected in the jungle from tree buttresses and in addition to yielding H. capsulatum

they were also usually positive for phlebotomus larvae). Soil samples are being collected periodically from the known positive sources in Madden Forest Preserve to determine the effect of seasonal variation on the recovery of H. capsulatum.

Nine soil samples collected on Barro Colorado Island from tree buttresses, in an ecological habitat apparently similar to Madden Forest, have not yielded the fungus. The importance of the tree buttress and the micro-ecology related to the isolations remain to be determined.

C. Studies of Clinical Histoplasmosis

The diagnostic assistance the Mycology Section has supplied to the Isthmian medical facilities has been limited almost exclusively to histoplasmosis. The diagnostic aids available for histoplasmosis are the collodion-agglutination test, the complement-fixation test, and facilities for isolation of the fungus from clinical specimens.

By September 1959 the Mycology Section has performed approximately 850 complement-fixation tests, using both histoplasmin and yeast phase antigens, and 600 collodion-agglutination tests. Over 60 cases of acute primary histoplasmosis have been confirmed by these serologic techniques. The policy has been to follow the serological titers of all positive sera when repeated sera on the patient can be obtained. The follow up of patients to obtain epidemiological histories has been vigorously pursued since the addition of other staff member. Histories obtained from patients with clinical histoplasmosis have resulted in the collection of samples of bat guano and soil samples in and around their homes; however, to date no isolations of H. capsulatum have resulted from these specimens. As in the case of skin test antigens, additional antigens are being considered for inclusion in the complement-fixation test for routine testing of sera.

Two disseminated cases of fatal histoplasmosis have occurred during the last 6 months: one was the second known fatal case in a native infant and the other was the only known fatal case in a Panama born adult. H. capsulatum was recovered from the antemortem blood and bone marrow of both patients.

D. Other Collaborative Studies

In addition to the sera obtained from Isthmian medical facilities we have performed serologic tests on sera from Guatemala, Republic of Panama, and Ecuador.

Several collaborative studies are underway with Capt. A.W. McFadden, ermatologist, Gorgas Hospital. A survey to determine the weekly mold count rom five different areas in Panama has been conducted for the past 9 months nd will continue for three more months when a correlation of mold counts nd respiratory allergic manifestations will be attempted.

Bats tested for the presence of the rabies virus by the Canal Zone ealth Bureau are being utilized by MARU in an effort to detect naturally ccurring histoplasmosis.

DIRECTION AND SUPPORT OF M.A.R.U.

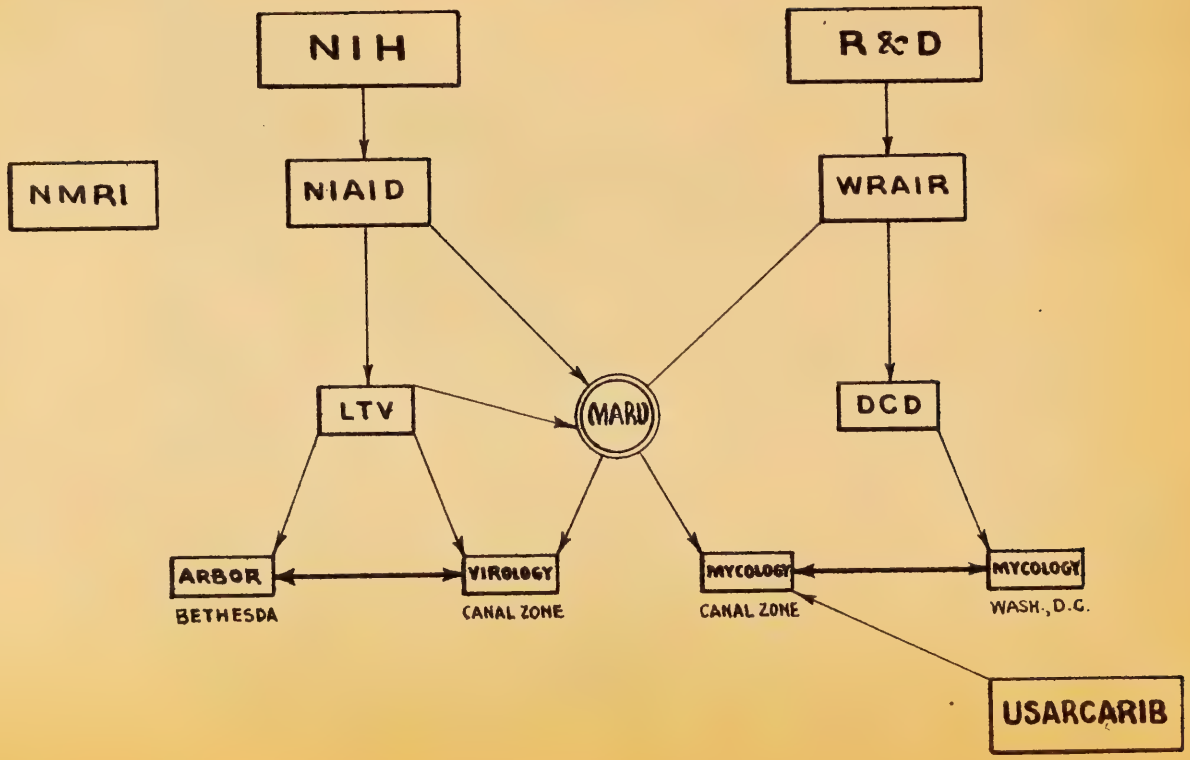
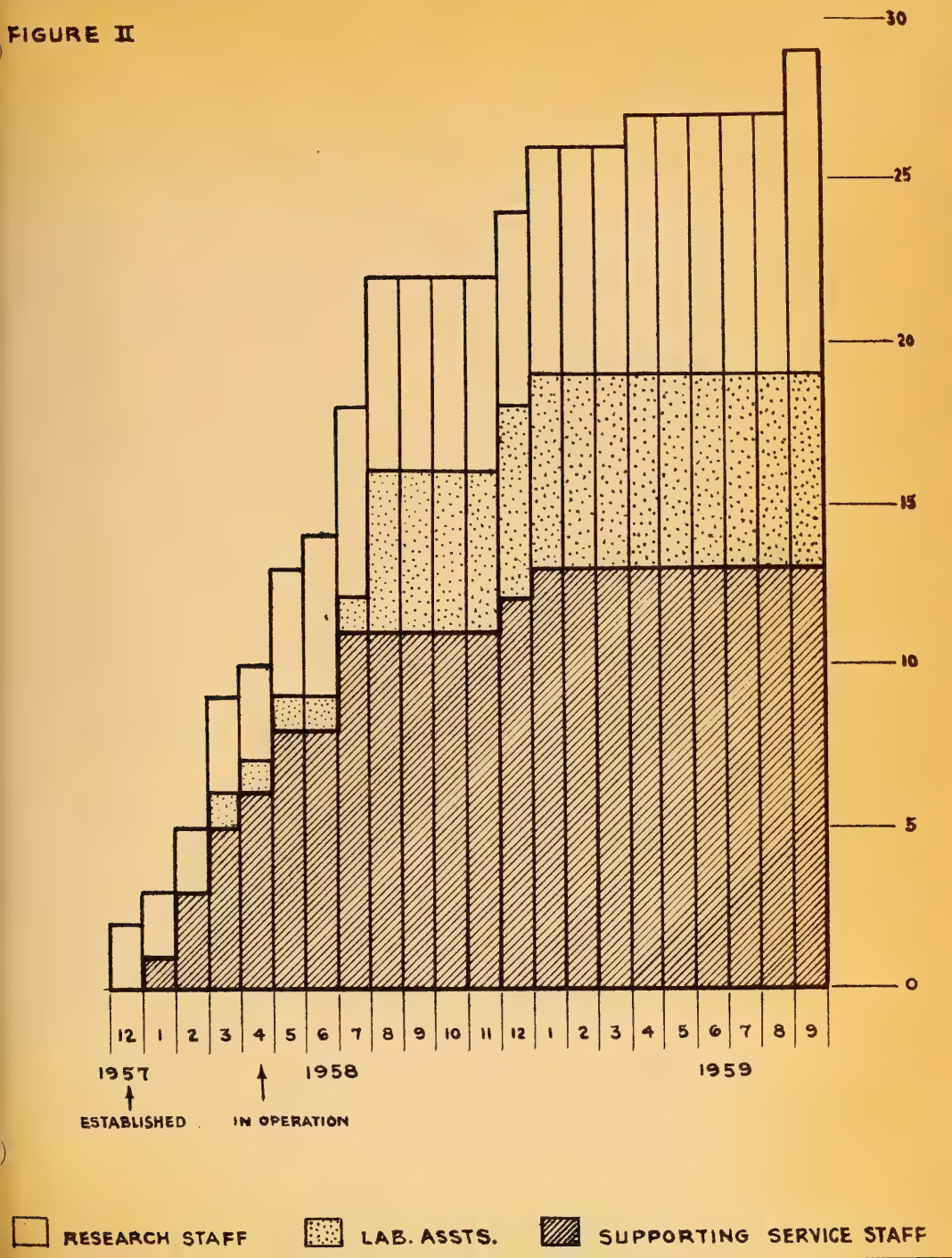


FIGURE I

EXPANSION OF LABORATORY PERSONNEL

FIGURE II



14 September 1959

MARU

TABLE I

MOSQUITO POOLS INOCULATED AT MARU DURING 1958 & 1959 STUDIES

Genus	# Species	# Pools
Aedes	6	100
Anopheles	3	20
Culex	10	124
Mansonia	5	150
Psorophora	4	10
Trichoprosporon	1	1
Uranotaenia	1	8
Wyeomyia	1	1
Mixed genera of mosquitoes	-	6
Phlebotomus	1	2
	32+	431

Notes: 1. 1958 & part of 1959 pools collected, identified, & processed @ GML.

2. Most pools contained 200 mosquitoes per pool.

3. At MARU all pools inoculated into SM & TC.

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MARU

TABLE 2

PRELIMINARY SUMMARY OF INITIAL SEROLOGIC TESTS

AS PART OF PACORA EEE STUDIES

	EEE: # tested			SLE: # tested **
	HI	NT	CF *	HI
Human	882	15	183	-
Horses	226	226	216	41
Bovine	128	5	-	-
Small Mammals	-	32	-	-
Bats	68	-	-	-
Reptiles	118	15	36	118
Birds	189	2	-	123
Total	1611	295	435	282

* Tested for WEE in addition.

** CFT & NT to be done.

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MARU

TABLE 3

PRELIMINARY RESULTS OF STUDIES ON BIRD SERA
FOR EEE & SLE ANTIBODY BY HI TEST
(Sera collected Oct-Nov 1958 @ Pacora, R. P.)

	EEE		SLE	
	# tested *	# positive ** HI titer ≥1:80	# tested *	# positive ** HI titer ≥1:80
Water Birds Herons & Ibis	15	4	12	2
Birds of Prey Vultures & Hawks	22	0	20	8
Passerine Birds Perching or Song	76	2	36	1
Miscellaneous Wild Birds	49	1	30	0
Domestic Birds Chickens & Pigeons	27	0	25	1
Total ***	189	7	123	12

* Serum acetone extracted & tested with 4-8u HA antigen.
** Complete inhibition of hemagglutination @ 1:80 dilution.
*** 17 additional birds tested for Ilheus, results incomplete.

1 September 1959

MARU

TABLE 4

PRELIMINARY COMPARATIVE EEE SEROLOGIC STUDIES

ON PACORA HORSE SERA

CFT vs. NT (in mice)

CF titer

	< 1/4	1/4	1/8	1/16	1/32	≥ 1/64	Total
NT (+)	12	7	12	9	1	1	42
NT (0)	51	2*	1*				54
	63	9	13	9	1	1	96

* Reactive with EEE & WEE by CFT

HI vs. NT (in mice)

HI titer

	< 10	10	20	40	80	160	320	640	1280	2560	>2560	Total
NT (+)				1	1	8	3	14	11	4	5	47
NT (0)	20	2	4	9	7	9	3	2	1	1		58

HI vs. CFT

	< 10	10	20	40	80	160	320	640	1280	2560	>2560	Total
CF + (≥1/4)				1	1	2	2	12	7	5	3	33
CF 0 (<1/4)	32		3	7	5	17	4	5	3	2	0	46

TABLE 6

PARTIAL RESULTS 1959-60 ENTEROVIRUS SURVEY OF PANAMANIAN INFANTS *

1959:	MAY		JUNE		JULY		TOTAL			
Age (in months)	# spec.	# (+) MK	# spec.	# (+) MK	# spec.	# (+) MK	# spec.	# (+) MK	% pos	
0-6	22	11	12	4	9	4	48	18	42%	30%
7-12	17	6	14	5	15	5	46	16	34%	
13-18	14	0	14	1	17	8	45	9	20%	27%
19-24	8	3	21	9	10	2	39	14	36%	
25-30	8	2	8	0	4	1	20	3	15%	21%
31-36	4	0	1	0	13	4	18	5	28%	
≥ 37	4	0	0	0	4	0	8	0	0	
Total	77	22 (29%)	70	19 (27%)	72	24 (33%)	219	65	30%	

* 70 specimens collected in August 1959, results incomplete.

Laboratory of Germfree Animal Research

National Institute of Allergy and Infectious Diseases

Summary statement of research progress, calendar year 1959

Program developments and trends:

Early in the year, the Section on Germfree Animal Studies of the Laboratory of Tropical Diseases was set up administratively as a separate Laboratory, as a part of the reorganization of NIAID. Among other things, this change seemed to reflect the feeling that the use of the germfree animal might be expected to increase and that it would find application in a wide variety of research interests within our Institute. There was no personnel change involved. During the year, two authorized additional positions were filled. A clerk-stenographer, GS-4, to take care of the clerical needs of the Laboratory and to serve as secretary to the Chief, was appointed. A histopathology technician, GS-7, was also added. It will now be possible to undertake, more fully and easily, corollary studies of the tissue responses in the various infections we are investigating in germfree animals, as well as the normal histology of the latter. As for future staffing plans, based upon impressions of major areas in which interest in germfree animal research is increasing, it is felt that eventual addition of a virologist and an immunologist or serologist would increase the effectiveness, and widen the scope, of our program. For example, it may well be that the combination of germfree animals and fluorescent antibody techniques would provide an excellent system for immunologic studies. Such personnel increases will, of course, depend upon whether, and when, additional laboratory space becomes available.

Significant program development occurred along two major lines during the year. First, the germfree mouse colony, initiated at the end of last year, increased to the point where these animals became available for experimental use. (In the past, the Institute's research program in this field had been limited to the use of germfree guinea pigs.) Secondly, and undoubtedly associated with the first, there has been an increase in the variety and number of cooperative projects either planned or underway with other Laboratories.

In order to undertake these studies we acquired an additional 4 new germfree animal units. We now have in operation a total of 12 of these units, 9 of which can be used for experimental purposes, although it is rarely that all are in use at one time. Studies involving disease agents may require at least 2 units at a time for a given experiment. Thus, if the current trend in increased interest continues,

The merits of expanding the laboratory and germfree animal unit space as envisioned in the contemplated acquisition of the rest of the 3rd floor in Bldg. 8, will become increasingly apparent.

In connection with the above, one also gets the impression that curiosity is being replaced by more genuine interest, outside the few germfree animal centers currently in operation. On the basis of personal experience, there appears to be more requests for seminars, workshops, working conferences, etc. on the current and potential use of these animals in research. For example, such a workshop has been requested of us by the Pathology Study Section early next year. This may well reflect an increase in grant activity in this area.

Significant scientific advances:

With regard to some of the projects underway, Mr. Phillips, in his studies of the lesions produced by Entamoeba histolytica associated with a variety of individual species of bacteria, has found that only mild to moderate infections are usually produced by some species, e.g., Escherichia coli and Streptococcus faecalis. On the other hand, a more acute ulcerative amoebiasis occurs when either Bacillus subtilis or Lactobacillus acidophilus is present. These findings may explain in part, the varied picture that infection with this parasite can present.

Germfree animals, especially guinea pigs, have notoriously enlarged ceca. On the "natural product" type of diet ordinarily used to raise this species germfree, this organ and its contents can comprise up to 40% of the animal's body weight. With the use of semisynthetic diets sterilized by irradiation, Dr. Horton has obtained germfree animals with an average cecal weight of less than 10% of the body weight, a figure which approaches that of the conventional animal. These studies may point out ways in which the lack of an intestinal flora affects the physiology of histology of the intestine.

In another type of study, we had shown earlier that the protozoan parasite, Trichomonas vaginalis, multiplied vigorously and produced large lesions when inoculated into the loose subcutaneous tissues on the back of germfree guinea pigs. However, for the most part, the organisms disappeared in conventional animals. Usually, a tiny fibrous nodule was all that remained, if anything, 2 weeks after inoculation. Recently, we have observed that if the germfree animals are orally contaminated with a single species of nonpathogenic, slowly-growing Micrococcus isolated from conventional guinea pig feces, and then are inoculated 2 weeks later, results resemble those obtained in the conventional animals. The presence of even a single "mild" species of bacterium appeared to stimulate seemingly nonspecific defensive mechanisms that were lacking or inadequate in the germfree animal. This animal-parasite system may provide a good device for the study of the role ordinary contamination may play in maintaining "natural"

...ease mechanisms.

As an example of the extra-Institute collaboration we attempt on a limited scale, we have just completed a study in which it was demonstrated that bacteria are not essential for the development of periodontal disease in mice. This appears to be true, also, for the development of calculus on the teeth. It is to be recalled that bacteria were required to produce dental caries in the rat

In collaboration with Mr. Rosenberger and Dr. Pond of the Laboratory of Tropical Virology, we have initiated studies on the behavior of viruses in the germfree host. It was noted that germfree mice survived somewhat better than did conventional mice following inoculation with either dengue type 2 virus or Japanese encephalitis virus. The differences, though not great, were significant. This finding would be of special biological interest if noted with other viruses, particularly in view of the fact that germfree animals have tended to be more susceptible than the conventional ones when challenged with other types of organisms.

One of the most intriguing things we've noted in this collaborative project has come from the use of germfree mouse kidney tissue culture. It was thought that tissue cultures from cells of animals which, for many generations, had not had to contend with a variety of infectious agents might possibly provide an especially favorable growth medium for viruses. If true, this might be more easily demonstrable with viruses that do poorly in conventional mouse cell lines. In parallel tests with dengue type 1 virus (the Mochizuki strain), marked differences in virus multiplication and production of cytopathogenic effects were noted between the germfree mouse kidney tissue cultures and those prepared from mice of the NIH colony. In the latter CPE was usually equivocal and titers ranged from 2.5 to 4.5 log $TCID_{50}$. In the germfree animal cell cultures, CPE was marked and titers as high as 7.5 log $TCID_{50}$ have been obtained. We are now using mouse kidney cells from the LCAR conventional colony, derived originally from the germfree mouse colony, to assess the possibility that a genetic or strain difference is involved. In our first simultaneous trial of cells from the 3 groups of mice, both conventional lines gave about the same result, but viral growth and CPE were much greater in the germfree mouse kidney. Thus, it would appear that the latter provides a good system for detecting dengue (1) virus.

As yet, of course, we do not know whether or not this is a general phenomenon or merely peculiar to this strain of virus. It is likely, however, that additional viral agents will be tried on germfree animal cells. If such cultures prove, in general, to be an especially sensitive system, their use for the detection of viral agents in suspect clinical material may develop. In anticipation of this we plan to increase our supply of germfree mice.

Other collaborative projects underway and to which we are able to devote some effort from time to time, as facilities permit, include: a study of the origin of anti-human blood group B agglutinins in chickens; an evaluation of the role of bacteria and diet in intestinal helminth infections; the response of germfree mice to injection of human infectious hepatitis materials.

Serial No. NIAID-116
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: The pathogenesis of amoebiasis

Principal Investigator: Bruce F. Phillips

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:
To study amoeba-bacteria relationships in the etiology, pathogenesis, and pathology of intestinal amoebiasis.

Methods Employed:
Germfree animals, and ex-germfree animals with induced contaminations with various known species of bacteria, are inoculated intracecally with trophic Entamoeba histolytica from culture. The intestines are examined grossly and microscopically for pathological change, and the nature and extent of lesions are determined for each amoeba-bacteria combination.

Major Findings:
Amoebic ulceration did not occur in germfree guinea pigs which received intracecal inoculation with bacteriologically sterile trophozoites of E. histolytica. Moderate amoebic ulceration occurred in ex-germfree guinea pigs harboring Escherichia coli, Aerobacter aerogenes, and Streptococcus faecalis, respectively, following amoebic inoculation. A more acute type of ulcerative amoebiasis developed in ex-germfree guinea pigs harboring Bacillus subtilis and Lactobacillus acidophilus, respectively, following amoebic inoculation.

Significance to Bio-Medical Research and the Program of the In...

The germfree experimental host provides the only practical means for studying the inherent disease-producing potential of the amoeba, or any enteric organism. The present investigation demonstrates that microbial synergism is important both in the development of enteric amoebic disease and in influencing the type and extent of lesions produced. This suggests a possible explanation for the diverse host-parasite relationships which characterize amoebic infection, although other factors are undoubtedly involved. In addition, the studies provide a stimulus for the study of other enteric diseases wherein microbial synergism may be involved.

Proposed Course of the Project:

The project will continue along the lines indicated above until information is obtained with several additional common types of bacteria including Staphylococcus and Proteus. It is hoped, also, that data may be obtained with the amoeba in association with an enteropathogenic species of bacteria.

Part B Included: Yes

Serial No. NIAID-110

PHS-NIH
Individual Project Report
Calendar Year 1955

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Phillips, B. P.: Parasitological Survey of Lobund Germfree Animals. Lobund Reports 3. (In press)

Honors and Awards relating to this project: None

Serial No. NIAID-IIH
1. Germfree Animal Research
2. Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Rearing germfree guinea pigs on diets sterilized by irradiation

Principal Investigator: Richard E. Horton

Other Investigators: John L. S. Hickey, Sanitary Engineering Branch

Cooperating Units: SEB-

Man Years (calendar year 1959):
Total: 1 1/6
Professional: 1/2
Other: 2/3

Project Description:

Objectives:

To evaluate commercial diets and semi-synthetic diets that are sterilized by irradiation and determine their adequacy to support and improve growth, development and reproduction in germfree guinea pigs.

Methods Employed:

Diets to be tested have been sterilized by the NIH Van de Graaff electron beam accelerator. Sterilized diet used in the rearing of germfree animals is aseptically transferred to the germfree chambers by employing the germicidal action of 3% peracetic acid. Various standard techniques for blood analysis, as well as growth curves, are used to evaluate effectiveness of diet.

Major Findings:

The minimum dose of 2 million rep has provided sterile batches of diet in every case. A three percent solution of peracetic acid solution with an exposure time of twenty minutes has been used successfully to decontaminate the surface of ampoules, plastic packages containing heat labile materials (e.g., vitamin solutions, irradiated diet and biological inocula). No contamination of a germfree unit has been traced to improper sterilization of these materials by the use of peracetic acid as the germicidal agent.

Attempts to rear germfree guinea pigs on an irradiated natural diet (e.g., commercial guinea pig pellets supplemented by yeast, ascorbic acid, thiamine and corn oil) have been unsuccessful. The majority of animals died between the 10th and 14th day of life due to inanition. Severe abdominal distension due to the accumulation of food material within the cecum and prolapse of the anus were a characteristic feature of animals reared on this diet. Development of the muscle and skeletal systems were markedly retarded.

The growth and development of germfree animals reared on an irradiated semi-synthetic diet (e.g., casein, corn starch, and oatmeal, plus necessary minerals and vitamins) has been better than animals previously raised on steam sterilized natural diet. Up to the present time, germfree guinea pigs have been maintained for 7 months on such a diet. The growth rate for the germfree animals appears to be less than that of the conventional animal maintained on the same diet.

Significance to Bio-Medical Research and the Program of the Institute:

Although germfree guinea pigs are being used in a variety of studies at the present time, they do not measure up to the conventional animal in many respects. Diet for the germfree guinea pigs as well as the sterilization method appear to be contributing factors. Improvements which lead to a more satisfactory animal for research seem worthwhile investigating. Standardized procedures for sterilizing by irradiation can be applicable to other germfree species. Biological response to irradiated diets can be observed in both conventional and germfree guinea pigs in the course of developing these procedures.

Proposed Course of the Project:

Studies to improve the growth rate of the germfree guinea pig during the early phase of life will be continued. Emphasis will be placed on diets composed of purified ingredients since they appear to give a growth response similar to that of conventional animals.

Part B Included: Yes

Serial No. NIAID-111

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project: None

Honors and Awards relating to this project:

Dr. Horton was invited to present his studies before the Animal Care Panel.

[illegible]
1. Biology
2. Parkman, Virginia

PHS-311H
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Biology of germfree animals

Principal Investigator: Richard E. Horton

Other Investigators: Walter L. Newton
William B. DeWitt, Laboratory of Parasitic Diseases

Cooperating Units: NIAID-121 J

Man Years (calendar year 1959):
Total: 1 1/6
Professional: 1/2
Others: 2/3

Project Description:

Objectives:

To obtain basic biological data on the germfree animal, with the view of determining in what characteristics it may differ from the conventional animal.

Methods Employed:

Comparative studies are made on germfree, specifically-contaminated, and conventional animals in such areas as hematology, blood chemistry, serology, gross and microscopic anatomy, growth curves, longevity, etc. Standard laboratory techniques are employed.

Major Findings:

1. On a semi-synthetic diet that was sterilized by irradiation (2×10^6 rep), there was little difference in the weight of the cecum and its contents between germfree and conventional guinea pigs: 9% versus 6% of the body weight, respectively. This is of considerable interest, in that the cecum and contents of germfree animals reared on so-called natural diets can comprise up to 40% of the total body weight. The hemoglobin and hematocrit values were essentially the same for both germfree and conventional animals. Fewer white cells were characteristically found in the peripheral blood of the germfree group.

3. Serum protein studies on 100 young and mature germfree and conventional guinea pigs (including conventional animals reared on the same sterilized diet as that given the germfree animals) revealed that:

a. Germfree animals had lower total serum protein levels than the conventional "contaminated" animals, regardless of which diet the latter were on.
b. Of the major serum protein fractions, the alpha globulins showed the greatest reduction in the germfree animals.
c. While the gamma globulins were lower in the germfree animals than in the conventionals maintained on the unsterilized stock diet, they were not different from those of animals maintained on the sterilized diet.

Similar studies underway on germfree mice have revealed no difference between the germfree animals and those of a mouse colony derived from the germfree colony and being maintained on the same sterilized diet, water, and bedding. However, both groups had levels of total protein and gamma globulins below those of mice from the conventional NIH colony, maintained on an unsterilized stock diet. The differences may have a nutritional basis (i.e., the effect of sterilization, or the composition of the diet). However, it is quite possible that the use of sterilized materials with our own conventional colony has reduced exposure to many infections which might affect gamma globulin levels. For example, we cannot find parasites which we know occur in the NIH colony.

Significance to Bio-Medical Research and the Program of the Institute:

Studies of this nature provide baseline data for current and future experiments that utilize the germfree animal. Also, they provide an opportunity to study the role that "normal" flora may play in such things as establishing hematological and serological values, affecting the architecture and permeability of the gut wall, and general growth, longevity, and fecundity.

Proposed Course of the Project:

Studies will continue along essentially the same lines. Where differences between the germfree and the conventional "contaminated" animals are encountered, efforts will be made to establish whether fundamental principles are involved, and whether a particular difference is worth further exploitation. Attempts to elucidate the apparent relationship between diet sterilization and serum protein levels will be pursued. Also,

further study of factors possibly contributing to the exceptionally enlarged cecum of the germfree guinea pigs maintained on a "natural" product diet is anticipated.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Warren, K. S. and Newton, W. L.: Portal and peripheral blood ammonia concentrations in germ-free and conventional guinea pigs. Amer. J. of Physiol., 197:3, 717-720, 1959.

Honors and Awards relating to this project:

Dr. Walter L. Newton was invited to be Chairman of the Germ-Free and Specific-Pathogen-Free Animal Session at the Animal Care Panel Convention in Washington, D.C. in October.

[illegible]

[illegible]

[illegible]

maintained within germfree chicks but not within conventional chicks, nor within healthy young adult human beings. In vitro studies have shown that products of blood group B active bacteria such as E. coli O_{86} B7 will coat human erythocytes so as to transform those of blood group O into B and those of blood group A into AB.

Significance to Bio-Medical Research and the Program of the Inst.

These studies may explain the mechanisms by which human beings afflicted with severe disease of their gastro-intestinal tract (e.g. cancer of the colon, incarcerated hernia, etc.) acquire a transitory B antigen on their erythrocytes.

Proposed Course of the Project.

If in vitro studies give positive results in the intact animal, the study can be terminated in a relatively short time. If the findings are negative, attempts will be made to increase intestinal permeability by physical or chemical means.

Part B Included: Yes

PHS-416
Individual Program Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Springer, G. F., Horton, R. E., and Forbes, M.: Origin of Anti-Human Blood Group B Agglutinins in White Leghorn Chicks. J. of Exp. Med. 110:2, 221-244, August, 1959.

Honors and awards relating to this project: None

Serial No. NIAID-114
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Oral disease in germfree mice

Principal Investigator: Walter L. Newton

Other Investigators: Paul N. Baer, Clinical Investigations Branch, NIDR

Cooperating Units: NIDR-

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/6
Other: 1/3

Project Description:

Objectives:

To ascertain whether periodontal disease occurs in germfree animals, with the resulting implications regarding the possible role of oral flora in the etiology of the disease.

Methods Employed:

The jaws of germfree and conventional mice of various ages reared on the same diet are examined grossly and microscopically for evidence of changes associated with this disease.

Major Findings:

Germfree mice showed alveolar bone loss and downward migration of epithelial attachment (characteristics of periodontal disease) just as did the conventional animals. The changes did not appear before the age of 4 months, and all animals had the disease after the age of 6 months. The findings indicated that, while the flora may aggravate the disease, they do not initiate the changes.

Of interest also was the fact that calculus was observed in germfree mice.

Significance to Bio-Medical Research and the Program of the Institute:

While this project was somewhat outside the general interest of our Institute, it is felt that we should cooperate with other groups in short-term projects that could show in clearcut fashion whether or not micro-organisms are etiologic agents in a particular disease. With regard to the findings of the project per se, the knowledge of which oral diseases depend upon, or are associated with, bacteria is of obvious importance. In this connection, it is of interest that previous studies have shown that dental caries does require a flora.

Proposed Course of the Project:

This project is essentially completed.

Part B Included: Yes

Serial No. NIAID-1th

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Baer, P. N., and Newton, W. L.: The occurrence of periodontal disease in germfree mice. J. Dental Res. (In press).

Honors and Awards relating to this project: None

Serial No. NIAID-[illegible]
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Behavior of parasitic protozoa in germfree [illegible]

Principal Investigator: Walter L. Newton

Other Investigators: Lucy V. Reardon, Laboratory of Parasitic Diseases
G. Robert Coatney, Laboratory of Parasite Chemotherapy

Cooperating Units: NIAID- 127 C
NIAID-130

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/6
Other: 1/3

Project Description:

Objectives:

To determine whether the presence of bacteria affect the course of, and response of the host to, protozoan infections such as Trichomonas or Plasmodium.

Methods Employed:

Germfree, monocontaminated, and conventional guinea pigs and mice are inoculated with axenically-reared T. vaginalis, or sterilely-obtained blood from mice infected with P. berghei. The inoculated animals are examined for the presence of lesions, parasitemia, time of death, etc.

Major Findings:

1. It has been shown previously that T. vaginalis, when injected subcutaneously, soon disappears in conventional guinea pigs, but multiplies and produces a severe lesion in the germfree animals. In our most recent experiments, germfree guinea pigs were infected orally with a single species of a slowly-growing, apparently nonpathogenic Micrococcus (derived from guinea pig feces) and challenged with T. vaginalis 2 weeks later. The

results were considerably more like those obtained with conventional animals, than with germfree controls. As suggested from the earlier data, "contamination" with living organisms, even a single species not obviously related to the challenge organism, apparently can stimulate or activate defensive responses of a seemingly non-specific nature.

2. Exploratory experiments comparing the course of *E. berghei* infections in germfree and conventional mice have failed to reveal any significant difference thus far.

Significance to Bio-Medical Research and the Program of the [illegible]

Projects of this kind can lead to a better understanding of the possible effects of the "normal flora" in maintaining a host's natural defensive mechanisms. It would appear that this, or a similar, system involving a host and an organism to which it is resistant in the conventional, but not the germfree, state provides a good device for such studies.

Proposed Course of the Project:

Additional experiments involving "conditioning" of germfree animals with other species of bacteria, dead bacteria, and perhaps such things as egg albumen, prior to challenging with *T. vaginalis* are planned. These may provide some indication of the specificity of this response.

Part 3 Included: Yes

Serial No. [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part E. Honors, Awards, and Publications

Publications other than abstracts from this project:

Newton, W. L., Reardon, L. V., and Delaney, A. M. A comparative study of the subcutaneous inoculation of germfree and conventional guinea pigs with two strains of Trichomonas vaginalis. Amer. J. of Trop. Med. and Hyg. (In press).

Honors and Awards relating to this project:

Dr. Newton was invited to present a lecture on germfree research at the University of Maryland.

Serial No. NIAID-116
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Studies on helminthic infections in germfree hosts

Principal Investigator: Walter L. Newton

Other Investigators: Paul P. Weinstein, Laboratory of Parasitic Diseases

Cooperating Units: NIAID-121 G

Man Years (calendar year 1959):
Total: 5/6
Professional: 1/6
Other: 2/3

Project Description:

Objectives:

To ascertain what effect the bacteria and other organisms normally present in the conventional animal have upon the course of helminth infections, and upon the host's response to these parasites.

Methods Employed:

Germfree animals are fed or inoculated with sterilized eggs, or axenically-reared infective larvae, of various parasitic helminths. The development of these parasites is followed by established procedures, and lungs, intestine, and other appropriate tissues are examined histologically for the study of host response. The findings are compared with those obtained in conventional controls.

Major Findings:

To supplement information obtained on the behavior of Nematospiroides dubius (a mouse helminth) in germfree and conventional abnormal hosts (the guinea pig), studies have been directed toward its behavior in the germfree normal host. Mature, fertile adult worms were obtained in germfree as well as conventional mice. Furthermore, numbers of worms, and egg out-

put by the females were consistent with what is customarily obtained in conventional mice. This indicated that the lack of bacteria appeared to have little or no effect on the development of the parasite from the infective larva to the adult stage. Attempts are now underway to complete the entire cycle in the germfree environment.

Significance to Bio-Medical Research and the Program of the Institute:

These studies can lead to an understanding of the influence bacteria may exert upon the establishment and course of helminth infections. The fact that bacteria, per se, are apparently not required for full development of some of these parasites opens the way for other studies in the areas of nutrition and parasite chemotherapy, in vitro cultivation, and physiologic studies. Also, opportunity is provided for the study of eggs and larvae in fresh (non-sterilized) feces without bacteria, and perhaps to ascertain factors contributed by the latter toward their proper development.

Proposed Course of the Project:

The comparative tissue response to the parasite in germfree and conventional mice will be studied. Efforts to complete the entire life cycle of the parasite in the normal fashion, but in a sterile environment, will be continued. If the germfree equipment and animals are available, attempts may be made to ascertain the role of bacteria in a certain parasite-diet relationship noted in conventional mice, and as reported in project NIAID-121 F.

Part B Included: No

Serial No. NIAID-117
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Response of germfree mice to injection of human infectious hepatitis materials

Principal Investigator: Walter L. Newton

Other Investigators: J. A. Morris, Division of Biologic Standards

Cooperating Units: DBS.

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/6
Other: 1/3

Project Description:

Objectives:

To determine pathologic and serologic responses of germfree mice to injection of clinical materials obtained from infectious hepatitis patients.

Methods Employed:

Two- to six-day old germfree mice are inoculated with either acute-phase hepatitis sera containing high titers of hepatitis hemagglutinins or with other sera free of detectable antibodies to these serum substances. At specific intervals the mice are sacrificed and examined for evidence of infection. Sera are examined for the presence of specific antibodies directed against hepatitis hemagglutinins; tissues are examined for evidence of specific antigen by the use of the fluorescent antibody technique, employing conjugated convalescent sera.

Major Findings:

In a limited study involving a total of 53 test and control germfree mice, no gross evidence of the presence of viral infection has been observed. With the use of the fluorescent

antibody technique for the demonstration of viral antigen a major obstacle still encountered is the presence, in convalescent hepatitis sera, of factors reactive in fluorescent systems with normal mouse tissue. This difficulty probably can be overcome through absorption with appropriate mouse tissue preparations. Thus far, however, it has not been possible to demonstrate in the tissues of the test mice an antigen specifically reactive with isothiocyanate-hepatitis serum conjugates. Information on the presence of hemagglutination inhibition antibody will be obtained when all the mouse sera in the study series are available for testing.

Significance to Bio-Medical Research and the Program of the Institute:

The study of human infectious hepatitis has been hampered by the fact that it has not been possible to isolate or demonstrate the presence of the virus except in humans. A significant contribution would be made if infection could be established in a laboratory animal.

Proposed Course of the Project:

Work will continue along the lines developed in the last six months, to explore thoroughly the potential value of employing gerafree animals, fluorescent antibody procedures, and hemagglutination technique, in detecting and isolating human hepatitis virus.

Part B Included: No

Serial No. NIAID-118
1. Germfree Animal Research
2. Pathogenesis
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: The use of germfree animals and tissues for the study of viruses

Principal Investigator: Walter L. Newton

Other Investigators: Charles Rosenberger and William L. Pond, Laboratory of Tropical Virology

Cooperating Units: NIAID-

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/6
Other: 1/3

Project Description:

Objectives:

To explore the possibility that germfree animals might serve as particularly suitable experimental hosts for certain aspects of virus study.

Methods Employed:

Comparative studies are carried out on the relative susceptibilities of germfree and conventional mice to infection with certain viruses. Standard virologic techniques are employed. Tissue cultures prepared from germfree animal tissues are also used.

Major Findings:

1. Several extensive attempts at isolating viruses from the germfree mouse colony have been made, with negative results. Thus far, the only evidence of a possible viral infection occurred in germfree mouse kidney tissue cultures. In two of many lots of mouse kidney preparations, some cytopathogenic effects were noted which were thought to be caused by an "infectious agent". The effect persisted for 3 passes in germfree mouse kidney cells and then disappeared. Inoculation

of suckling mice with these suspicious materials caused no observable disease. We can tentatively conclude that an infectious agent may have been present and, if so, it may have come from the germfree mouse colony.

2. In comparative studies on the course of infection of dengue type 2 virus and Japanese encephalitis virus in germfree and in conventional mice, some small but significant differences were observed in survival. Of interest was the fact that, in contrast with much of the experience with germfree animals, the latter behaved as though they were more resistant to these viruses than were the conventional animals. Death of the germfree mice was usually delayed a few days, and often the number of survivors was significantly greater than was true of the conventional mice.

3. Studies are underway to determine whether tissue cultures prepared from cells of animals that had been reared under sterile conditions for many generations might provide a better growth medium for certain viruses which grow poorly in conventional animal cell lines. Significant differences have been observed rather consistently in experiments with dengue type I virus (the Mochizuki strain). In germfree mouse kidney tissue culture, marked cytopathogenic effects and titers as high as 7.5 log $TCID_{50}$ have been obtained. In kidney tissue cultures prepared from conventional mice, CPE was equivocal and titers ran only from 2.5 to 4.5 log $TCID_{50}$. Also, in one trial it was observed that titer and degree of CPE increased when the supernate from normal mouse kidney tissue cultures containing the Dengue-M virus was transferred to germfree mouse kidney tissue culture, and decreased when returned back to normal mouse kidney tissue cultures. It would appear that the germfree mouse may provide a good tissue culture system for detecting dengue virus.

Significance to Bio-Medical Research and the Program of the Institute

These studies will prove to be of considerable value if they result in the development of improved methods for detecting the presence of, and for growing, viruses for which present methods are not satisfactory. Also, the study of viruses in whole animals without possible influences of other concomitant infections might provide additional insight into virus-host relations.

Proposed Course of the Project:

It is likely that most of the emphasis in the near future will be placed on studying the behavior of other viruses in germfree animal cells to see if the observed differences can be repeated. If possible, we would also like to initiate studies with the view of ascertaining the basis for these differences.

Part B Included: No

additional personnel in order to preserve the vigor of the Bethesda program.

The emphasis in the Laboratory is on basic research, largely because of the interests and previous orientation of the principal investigators in the Sections that became part of LPD. There has been no major shift in program, but it is anticipated that the scope of the Laboratory will broaden somewhat when personnel vacancies in the Section on Helminthic Diseases are filled.

The Section on Clinical Investigations is set up, administratively, with a nominal head who is assigned and responsible to the Laboratory of Clinical Investigations. It is for this reason that no description of this Section's projects is given in the attached reports; this would merely be duplication. What we do need is a clearer alignment of responsibility and authority for the clinical studies within this framework. The initiative for a clinical study sometimes arises in the laboratory and, subject of course to its appraisal as a worthwhile endeavor, should receive support from the clinical investigators. The converse is already true. Alternatively, and perhaps wisely, occasional clinical projects may be conducted by LPD personnel elsewhere than at the Clinical Center. Here again, however, we need authority and flexibility in order to initiate such studies, within reasonable limits of personnel and budget.

The major shifts in personnel have been due to the assignment of Dr. W. T. Haskins (Scientist Director, organic chemist) from Helminthic Diseases to the Rocky Mountain Laboratory, the retirement from the same Section of Dr. Myrna F. Jones (Medical Parasitologist, GS-11), and the appointment of Dr. Louis S. Diamond to fill a vacancy in the Section on Protozoal Diseases. It has been decided to substitute for Dr. Haskins' position a lower-grade scientist and a subprofessional job. This was done to adjust the previously disproportionate relation between the professional and supporting staffs in the Section on Helminthic Diseases. With the retirement of Dr. Jones, work on helminthic infections in germ-free animals has been prosecuted by Dr. Weinstein, whose own work on nematode cultivation is particularly suited to extension to germ-free research. The lower-grade professional vacancy will be used to support him, while Dr. Jones' job will be used to initiate studies on filariasis. We are currently looking for qualified personnel for these two vacancies.

The appointment of Dr. Louis S. Diamond has resulted in some renewals and shifts of interest. He has resumed studies on the problem of cultivation of endoparasitic protozoa, and has initiated work on the cytochemistry of amebae and flagellates using fluorochrome dyes.

Because of the fact that the Section is understaffed, and because maintenance of strains requires a great deal of time and labor, we have arbitrarily decided to reduce the number of strains maintained and to explore freezing techniques as a means of storage.

Significant scientific advances:

The importance of nutrition in relation to self-cure and chemotherapy of helminthic infections has been shown in studies on mice infected with schistosomes and various nematodes and cestodes (Projects # 121-F, 121-K). Nicotinic acid deficiency enhances the susceptibility of mice to Schistosoma mansoni infections, but the worms are stunted and incapable of normal egg-production. Folic acid deficiency, with or without B_{12} deficiency, does not alter susceptibility of the host, although infection aggravates the resultant anemia, but decreases egg-production of the worms. Mice on a semi-synthetic diet alone or supplemented with vitamin B_{12} apparently completely lost their oxyurid or tapeworm infections, or harbored stunted Hymenolepis nana, as compared to controls on a Purina pellet diet. Of great significance is the finding that treatment with stibophen produced much higher cure rates of S. mansoni infections in mice on an enriched semi-synthetic diet as compared to mice on a commercial diet. It is because of these very promising leads that we have initiated the Puerto Rican study on human beings infected with S. mansoni (121-L).

The interrelation of nutrition and parasite physiology is indicated in the finding that Nippostrongylus muris, cultivated in vitro in the presence of low concentrations of vitamin B_{12}, accumulates a reddish pigment in its coelomocytes. The chemical identity of this pigment is being investigated. Should further work reveal that it is indeed vitamin B_{12}, this would be the third instance of the concentration of this vitamin in helminths, and an indication of a subtle biogenetic mechanism.

The successful development of a helminth in a germ-free environment and in germ-free animals (121-G) opens the way for comparative study of the intricate factors involved in the host-parasite relation.

The importance of liver disease in parasitic infections has led to a series of studies of general medical interest on the mechanism of ammonia toxicity (121-M). It has been demonstrated that hypoxia markedly increases the toxicity of ammonia in mice. A large number of other factors have been studied but have had only slight effect.

The development of a method for testing molluscicides against snail eggs rather than adult snails (121-C) is an important technical

improvement in this field. Also, the finding that ultraviolet light rapidly inactivates sodium pentachlorophenate is of great importance in explaining failures in the effectiveness of this compound under certain field conditions. Since NaPCP has been demonstrated to be about 10X as effective against snail eggs compared to adult snails, this suggests that low concentrations of the chemical may be useful in maintaining bodies of water snail-free.

Considerable progress has been made towards an understanding of the calcareous corpuscles and phospholipids of various cestodes and trematodes (123-A).

Studies on the pathologic physiology of various protozoan infections, as revealed by histochemical techniques, have revealed that infections with Trypanosoma congolense, T. equinum, T. equiperdum, and Bartonella muris show individual peculiarities with respect to liver glycogen and lipid, and that such nonspecific factors as anoxia and adrenal involvement cannot be the only pathogenetic mechanisms. They indicate the possibility that the parasites produce specific substances that influence the observed changes. This has an important bearing in relation to research on chemotherapy and immunology (123-B).

The basic studies on the biochemistry of mitochondria (123-C) which developed from earlier work on the effect of sodium pentachlorophenate on snails have been continued, with the demonstration that isolated mitochondria have an appreciable endogenous respiration in the absence of added substrate, and that this is linked with their stability. Only certain reactions of isolated mitochondria have been shown to be affected by the age of the animal; the oxidation of beta-hydroxy-butyrate is impaired with age, but not the subsequent oxidation of acetoacetate. These studies now have a parasitologic orientation as well; it has been shown that the mitochondria isolated from rats parasitized with T. equinum are deficient in oxidative phosphorylation.

The work on toxoplasmosis (127-A) has shifted to emphasis on the chronic stage of infection and on the cyst. The nature of the cyst wall, which is of considerable importance in relation to exacerbations during chronicity, has been shown by the electron microscope to be a complicated structure rather than a simple membrane. The occurrence of congenital transmission during chronicity has occurred most frequently with parasite strains that produce abundant cysts and also late parasitemias. Such parasitemias are seen in mice, rabbits, and guinea pigs, but not in rats; in the rat, cortisone occasionally

induces parasitemia. The injection of hyperimmune serum into chronically infected mice results in some increase in the number showing parasitemia. This may be of clinical importance in relation to the effect of re-exposures to the parasite on already existing chronic infections. We have made additional progress in evaluating the usefulness of the hemagglutination test in various animals. The sera of monkeys, pigeons, and sheep give good results as compared with the dye test, while sera of rats and dogs do not. Of various fractions prepared from the hemagglutination antigen, one appears to be stable on storage.

The axenic cultivation of Entamoeba terrapinae for the first time, and of E. invadens, in an improved medium without particulates, represents an important step towards the goal of cultivating E. histolytica without associated microorganisms (127-B). Some work on E. histolytica has indicated that the mammalian embryo extracts useful for E. terrapinae and E. invadens may be of value as substitutes for living cells. The usefulness of fluorochrome stains for identifying DNA and RNA in protozoan cells has also been exploited in identifying DNA in the endosome of amebae and differentially staining the parabasal body of a trichomonad. One of the most promising developments in this project is the development of cryogenic techniques for storing parasitic protozoa. This will eventually reduce the burden of strain maintenance and also preserve the characteristics of strains.

The continued work on a hemagglutination test for trichomoniasis (127-C) has given consistently good results with animal sera prepared against lysed Trichomonas antigens. This test now requires trial with human sera and vaginal exudates. Continued work on enzyme systems of T. vaginalis has resulted in a partially purified malic dehydrogenase. This biochemical work on an anaerobic protozoan is significant in that it may eventually lead to an understanding of general methods for the anaerobic reoxidation of reduced pyridine nucleotides.

The work on Pneumocystis and Sarcocystis (127-E) that we have instituted represents a new field of study. The development of some techniques for handling these organisms now makes further work possible on their biology and pathogenesis.

Project No. [illegible]
1. Parasitology - General
2. Office of the Chief
3. Research, Administrative

HSN-2.1
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Administration and research planning and coordination

Principal Investigator: Leon Jacobs

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1959):

Total:	2½
Professional:	½
Other:	2

Project Description

This project furnishes supervisory and administrative service to all research projects in the Laboratory, as follows:

Overall evaluation of research plans and initiation of field projects; integration of laboratory research activities with clinical studies at the Clinical Center; editing scientific and technical reports and manuscripts; preparation of reports, budget estimates, and exhibits; supervision over personnel, travel, correspondence, and maintenance; requisitioning and supervision of equipment and supplies; maintenance of reference library, etc.; and consultatory services to individuals, academic and other organizations, and liaison activities with other branches of the Service and the Federal Government.

Research projects are reviewed quarterly on a formal basis and informally in continual discussion with staff members. In consultation with Section heads, changes in research programs are discussed and initiated. The emphasis is placed on fundamental aspects of medical parasitology; extensions of projects into the fields of clinical or practical preventive medicine are attempted only when the laboratory work has progressed to a point where a sound basis exists.

An effort has been made to analyse the personnel of each Section with reference to the programs being or to be presented. Efficient utilization of professional and subprofessional personnel has been accomplished in one Section by rearrangement of the number of jobs in each category. Further analysis of the other Sections is under way.

Part B included - No

Series No. [illegible]
1 Parasitic Diseases
2 Research Project
3 Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Host-parasite reactions in [illegible] rodents / laboratory animals

Principal Investigator: Louis J. Olivier

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1959)

Total:	[illegible]
Professional:	[illegible]
Other:	None

Project Description:

Objectives:

To study the [illegible] of [illegible] in laboratory animals to learn how resistance to infection may be enhanced and reduced; to learn how the host defense mechanisms act to [illegible]; and to study variation in resistance of host strains.

Methods Employed:

Infect animals in such a way as to [illegible] treat with hormones, antigens, [illegible], radiation, [illegible]; observe the host and parasites, and determine by examining [illegible] and worms, and by the study of tissue sections. The degree of resistance and the host tissue reaction.

Major Findings:

The project has been relatively inactive during the year due to diversion of personnel to other projects. It is expected that the project may be renewed when an existing vacancy is filled.

Significance to the Program of the Institute:

Problems of resistance and immunity are basic to understanding of host-parasite relations. Invasiveness of parasites and host reactions to parasites are important clues to an understanding of the pathogenicity of worms.

Proposed Course of the Project:

To continue study of the effects of hormones on worm infections.
To study the acquisition of resistance to schistosome infection.
To study host-parasite relations in filarial infections.

Part B included - No

Serial No. NIAID-121-B
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Role of helminths in the causation of cancer

Principal Investigator: Louis J. Olivier and Elmer G. Berry

Other Investigators: None

Cooperating Units: Pathological Anatomy Branch, National Cancer Institute, NCI-525

Section on Cancer Products, Laboratory of Bacterial Products, DBS

Man Years (calendar year 1959):

Total: ½
Professional: ¼
Other: None

Project Description:

Objectives:

To test whether worm parasites can induce malignant growths in their hosts. To investigate the possibility that liver tumors of rats caused by Taenia taeniaeformis infection are due to a virus. To test whether Schistosoma haematobium infection in hamsters causes tumors of the digestive or urinary tract.

Methods Employed:

Taenia taeniaeformis. Infected rat tissue and worm material are inoculated into tissue cultures in an attempt to pass a possible cancer-producing agent.

Schistosoma haematobium. Hamsters are infected with the worms in an attempt to induce malignant growths.

Major Findings:

The Taenia experiments failed to uncover evidence that the rat tumors were virus-induced. A latent rat virus was discovered, however. The study has been terminated.

The schistosome study is still under way. No new animals are being infected. No tumors have been induced. A few hamsters are still alive. The tissues are being studied.

Significance to the Program of the Institute:

The relation of parasitic worm infections to malignancy is a subject that has been mentioned from time to time in the literature. Most of the suspicion stems from observational data, and is far from definitive. Continued investigation should increase our knowledge of the significance of worm infections and provide new information concerning factors which may predispose an individual to cancer, either by themselves, or in concomitant infections.

Proposed Course of the Project:

Dependent on the availability of personnel, it is proposed to study the influence of worm infections on tumors produced by known viral carcinogenetic agents.

Part B included - Yes

Serial No. NIAID-121-B

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than Abstracts from this project:

Kilham, Lawrence and Olivier, Louis J. 1959 A latent virus of rats isolated in tissue culture. Virology 7 (4): 428-437.

Honors and Awards relating to this project: None

Serial No. NIAID-121-C
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Destruction of molluscs by chemical means.

Principal Investigator: Louis J. Olivier

Other Investigators: Willard T. Haskins (until June 30, 1959)

Cooperating Units: Laboratory of Viral Products, DBS.

Man Years (calendar year 1959):

Total:	2 1/2
Professional:	1 1/4
Other:	1 1/4

Project Description:

Objectives:

To discover more effective means for killing snail vectors of schistosomiasis. To study the mode of action of chemicals used as molluscicides. To develop more effective means for study of snail poisons in the laboratory.

Methods Employed:

Snail vectors, or their eggs, are exposed to chemicals and the destructive efficacy of the chemicals is recorded. The optimal conditions for use of chemicals against snails are sought. By quantitative methods the optimal duration of exposure, concentration, temperature, etc. are determined.

Major Findings:

Snails exposed to non-lethal concentrations of sodium pentachlorophenate (NaPCP), the chemical of choice for destruction of fresh--water snails, were rendered far less productive than controls and some ceased egg-production. The eggs produced under such conditions usually failed to develop. Thus, concentrations of the chemical far below those used in the past may control snail

populations by rendering the adults unproductive.

Ultra-violet light, is highly destructive to NaPCP and can effectively remove its toxicity for snails. This may explain failures with this compound encountered in the field under certain conditions.

Newly laid eggs are far more susceptible to NaPCP than older eggs. The chemical acts very rapidly on these eggs.

NaPCP is about ten times as active against the eggs as it is against adults of the same strain. This suggests the use of smaller concentrations of the chemical with the intention of controlling the snails by interfering with reproduction of survivors or newly introduced individuals after destruction of the majority of adults by larger concentrations of the chemical.

NaPCP is far more active against snail eggs than is copper sulfate.

A method has been developed for screening of chemicals as molluscicides, using snail eggs rather than adult snails. The method permits more accurate and economical experiments since the eggs are cheaper to produce and more readily handled in large quantity. There is reason to believe that the results obtained will be more reliable not only because larger numbers of test animals may be used but because the eggs are probably more uniform than laboratory-reared adult snails. Screening is not planned but the method may be useful in other laboratories interested in this procedure.

Significance to the Program of the Institute:

For the present, at least, the best means to control schistosomiasis is interruption of transmission from man to man by destruction of the snail intermediate hosts. The molluscicides now available are not ideal. The project attempts to find better and more economical ways to use the available molluscicides. The evidence from the work done so far suggests the possibility that more efficient use may be made of molluscicides if they are directed against the snail eggs than against the adults. The project puts the Institute in a better position to take part in measures directed against the schistosomes which are rapidly coming to prominence as dangerous parasites of man.

Proposed Course of the Project:

To continue with efforts to standardize the laboratory method for study of molluscicides with the aim of perfecting a method for highly analytical quantitative work with snail poisons and also with the aim of providing laboratories interested in chemical screening with a better tool. To investigate the action of chemicals already studied upon other species of vector snails. To try, under the same conditions, other molluscicides of high promise so that there may be accurate and quantitative data by which molluscicides can be compared.

Part B included - Yes

Serial No. NIAID-121-C

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Olivier, L. and Haskins, W. T.: The effect of low concentrations of sodium pentachlorophenate on the fecundity and egg viability of Australorbis glabratus. To be published in the American Journal of Tropical Medicine and Hygiene.

Olivier, L. and Barbosa, F. S.: Survival and weight loss of Australorbis glabratus kept at controlled relative humidities in the laboratory. To appear in the proceedings of the Sixth International Congress on Tropical Medicine and Malaria.

Olivier, L.: Factors affecting the survival of aestivating pulmonate vectors of schistosomiasis. To be published in a book celebrating the anniversary of Dr. Caballero y Caballero.

Honors and Awards relating to this project:

Dr. Louis J. Olivier served as consultant to the WHO from November 15, 1958 to January 22, 1959 in order to conduct a survey of bilharziasis in Iran and plan a program of WHO participation on bilharziasis research there.

Dr. Louis J. Olivier served as consultant to the WHO in Geneva in order to participate in a Study Group on Molluscicide Research which met from February 9-13 and to prepare a document on the relation of irrigation to bilharziasis from February 13-28.

Serial No. NIAID-121-D
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigation of intermediate hosts and vectors of human diseases caused by worms

Principal Investigator: Elmer G. Berry

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1 1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives:

To investigate the distribution, life history, and ecology of snail intermediate hosts and to develop a rational and useful system of classification and means of identification.

Methods Employed:

Colonies of known snail vectors are maintained in the laboratory where they are available for intensive study. Suspected vectors are exposed to infection to evaluate their ability as transmittors of schistosomes in the field.

Major Findings:

Continued progress has been made toward the discovery of criteria useful for the accurate identification of the snails which serve as the intermediate hosts of Schistosoma mansoni

and Schistosoma haematobium. In addition, a monograph on some of the vectors of schistosomiasis is in preparation.

Significance to the Program of the Institute:

This knowledge is essential if one is to understand the epidemiology of worm infections transmitted by vectors and if one is to devise adequate control procedures.

Proposed Course of the Project:

Continue anatomical studies of snails in order to make possible their accurate identification. Study variation among snail strains with respect to susceptibility to infection with schistosomes.

Part B included - Yes

Serial No. NIAID-121-D

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project: None

Honors and Awards relating to this project:

Dr. Elmer G. Berry served as a member of a WHO Study Group on Molluscicide Research which met in Geneva February 9-13.

Dr. Elmer G. Berry served as advisor to the WHO in Geneva from February 16-March 6.

Dr. Elmer G. Berry served as consultant to the WHO on the control of bilharziasis in Africa from October through December.

Serial No. NIAID-121-E
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development of methods for the cultivation of parasitic helminths in vitro and the determination of the nutritional requirements of such organisms in vitro

Principal Investigator: Paul P. Weinstein

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1 1/2
Professional:	1/2
Other:	1

Project Description:

Objectives:

To develop media suitable for in vitro cultivation of helminths throughout their life cycle, and to obtain an understanding of their specific nutritional requirements.

Methods Employed:

Worm eggs or larvae are isolated bacteria-free, are inoculated into culture media to be tested for growth-promoting properties, and are observed for development and differentiation.

Major Findings:

In experiments evaluating the effect of vitamin B_{12} upon nematode growth, it was found that a reddish pigment was specifically concentrated in the coelomocytes of Nippostrongylus muris.

The pigment can be visually observed in these cells when B_{12} is present in as low a concentration as 0.05 microgram per ml medium. Vital dyes e.g. neutral red are not concentrated in these cells even in concentrations as high as 50 micrograms per ml medium. The chemical identity of the pigment is being investigated.

The pigment concentration has also served as a convenient marker by which to follow the migration and development of these normally colorless cells which are ordinarily very difficult to observe in the living worm. The function of coelomocytes in nematodes has previously not been elucidated.

The addition of various vital dyes to the culture medium has in turn led the way to studying their deposition and mechanism of excretion by the worms during normal growth, hitherto not possible using *in vivo* methods.

Significance to the Program of the Institute:

These findings bear on the nutritional and excretory processes of parasitic helminths. Such studies may ultimately help explain some clinical, pathological and immunological effects of worm infections, and may enable better diagnosis and treatment.

Proposed Course of the Project:

Work will continue on the nature of the pigment found in coelomocytes of nematodes exposed to B_{12} to determine whether this is a selective concentration of B_{12} itself, or whether the worm is forming a pigment of other chemical nature in response to B_{12}.

In addition, the growth response to other nutrients is being studied, as well as factors which are involved in mating of nematodes.

Part B included - Yes

Serial No. NIAID-121-E

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Weinstein, Paul P. and Jones, Myrna F. Development *in vitro* of some parasitic nematodes of vertebrates. Ann. N. Y. Acad. Sci. 77: 137-162, 1959.

Honors and Awards relating to this project:

Dr. Weinstein was reappointed as an Assistant Editor of the Journal of Parasitology.

Dr. Weinstein was appointed an Instructor and taught in the Invertebrate Zoology Course at the Marine Biological Laboratory, Woods Hole, Mass.

Serial No. NIAMD-121 F
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Nutritional aspects of helminthiasis

Principal Investigator: William B. DeWitt

Other Investigators: Paul P. Weinstein

Cooperating Units: None

Man Years (calendar year 1959):

Total	1 3/4
Professional:	1/2
Other:	1 1/4

Project Description:

Objectives:

To determine the role of nutritional factors in the disease processes associated with helminthic infections, their effects on the development of the parasite, their possible influence on the development of resistance to reinfection and their effects on host susceptibility.

Methods Employed:

Animals maintained on natural or purified diets either deficient in or enriched with specific components are exposed to various helminths. The influence of the diet and its consequences on the host and on the parasite are studied.

Major Findings:

A. Schistosoma mansoni. A diet deficient in nicotinic acid increases the susceptibility of mice to S. mansoni infections so that more than twice as many worms can be recovered from the deficient mice as from mice given a complete ration. The worms recovered at necropsy of the nicotinic acid-deficient mice are found

to be stunted and incapable of normal egg production. It was found that folic acid deficiency either alone or associated with vitamin B_{12} deficiency does not alter the susceptibility of the host to S. mansoni; however, in with the nicotinic acid deficiency, the ability of the parasites to produce eggs was sharply decreased. The folic acid - B_{12}-deficient mice developed an anemia which became more severe due to the development of the schistosome infection.

B. Nematospiroides dubius. Egg production, yield of adult worms, and worm size were smaller in mice on a semi-synthetic diet alone or supplemented with vitamin B_{12} than in mice on a Purina pellet diet.

C. Pinworms and tapeworms. Mouse oxyurids and the tapeworm Hymenolepis nana completely ceased egg production and were presumably eliminated from mice on the complete semi-synthetic diets, while infection with these helminths remained at a relatively high level in mice on the Purina diet. Mouse growth on the semi-synthetic diet was equivalent to or better than that on the Purina diet.

Significance of this Program to the Institute:

Debilitating helminthiases develop frequently in human populations living on deficient diet. In order to understand the clinical problems adequately, it is necessary to assess the role of the diet and of the parasites in producing debilitations. The problem of treatment, prevention, and control of parasitic infections can be met more effectively if these relations are understood.

Proposed Course of the Project:

Further studies are planned to determine the factors in the semi-synthetic diet which are either lacking or are acting to achieve the effects described above. The use of germfree animals may aid in this.

Part B included - No

[illegible] No. [illegible]
Parasitic Diseases
Helminthic Diseases
Beltsville, Maryland

PHS-ARS
Individual Project Report
Calendar Year 1969

Part I.

Project Title: The development of helmi[illegible] in guinea animal[illegible]

Principal Investigator: Paul T. Weinke[illegible]

Other Investigators: Walter L. Newton, [illegible]rector of Veterinary Animal Research, [illegible]

Cooperating Units: Laboratory of Parasi[illegible]nimal Research, Inc. Central Pk. PHS/ID-[illegible]

Man Years (calendar year 1969):

Total:	1 1/4
Professional:	3/4
Other:	1/2

Project Description:

Objectives:

To establish helminth infections [illegible] guinea animals to [illegible] problems involving host-parasite relati[illegible] nutrition, immune re[illegible] sponse and pathology and [illegible] absence of other [illegible] eliminating microorganisms.

Methods Employed:

[illegible] (as named) to the investig[illegible] age under sterile co[illegible] ditions and ages and sexes are also [illegible] from natural [illegible] and reared exotic; these are used [illegible] test guinea animal[illegible].

Major Findings:

Parasite-free test animals [illegible] infected with [illegible] gracilis dubius larvae [illegible] commercially and the source of [illegible] infection so far has closely paralleled that in conventional control mice. Previous studies have shown that this nematode will not develop to the adult stage in the conventional guinea pig (an abnormal host), but will do so in the germfree guinea pig.

Significance to the Program of the Institute:

The successful infection of a normal host with a nematode parasite under germfree conditions, together with the finding that this organism will develop in a germfree abnormal host but not in its conventional counterpart presents a promising tool for studying factors involved in natural resistance, in host specificity, as well as in immune response.

Proposed Course of the Project:

The comparative pathology between conventional and germfree mice is to be studied. It is also planned to attempt to establish the infection on a continuing basis in the germfree environment through successive generations of host and parasite and to follow the course of the infection under such conditions. Nutritional factors relating to infection will be one of the first areas to be investigated.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Newton, Walter L., Weinstein, Paul P., and Jones, Myrna F. A comparison of the development of some rat and mouse helminths in germfree and conventional guinea pigs. Ann. N.Y. Acad. Sci. 78: 290-306, 1959.

Honors and Awards relating to this project: None

Serial No. NIAID-121-3
1. Parasitic Diseases
2 Helminthic Diseases
3 Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pathological physiology of worm infections.

Principal Investigator: Kenneth S Warren

Other Investigators: William B. DeWitt

Cooperating Units: None

Man Years (calendar year 1959):

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives:

In the course of previous investigation: portal hypertension and esophageal varices were found in mice infected with Schistosoma mansoni. The pathology and liver function test derangement were similar to those encountered in humans. Present studies have two major objectives: 1. To determine the specific etiology of portal hypertension in mice infected with S. mansoni. 2. Since portal hypertension has never before been produced consistently in any animal an attempt is being made to do so in dogs and monkeys. New surgical techniques could then be developed and changes in liver physiology could be investigated

Methods Employed:

Changes in the portal circulation of mice, dogs, and monkeys are studied at intervals following infection, by dissection, by measurement of portal pressure, by X-ray following injection of colored latex into the system, and by the study of liver tissue sections.

Major Findings:

A syndrome that closely resembles hepato-splenic schistosomiasis in humans was reproduced in mice. Dogs and monkeys have been infected for 2 years with S. japonicum and S. mansoni respectively in an attempt to reproduce this syndrome in a larger animal. Thus far neither portal hypertension nor collateral circulation have been produced.

Significance to the Program of the Institute:

Schistosome infections in laboratory animals provide a means to study cirrhosis, collateral circulation, and ammonia metabolism. Portal hypertension has never been consistently produced in an animal before, and this is of interest in the entire field of medicine.

Proposed Course of Project:

Continue study on the pathology of schistosome infections in an attempt to determine the cause of the development of portal hypertension in mice. Attempts are being made to reproduce the hepato-splenic syndrome in dogs and monkeys.

Part B included - Yes

Serial No. NIAID-121-I

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

DeWitt, W. B. and Warren, K. S: Hepato-splenic schistosomiasis in mice. Am. J. Trop. Med. & Hyg., 8: 440-446, 1959.

Warren, K. S and DeWitt, W. B.: Esophageal varices in mice infected with Schistosoma mansoni. Proc. 6th Internat. Congr. Trop. Med. & Malaria, in press.

Serial No. NIAID-121-J
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Serum protein studies on germfree animals infected with various parasites

Principal Investigator: William B. DeWitt

Other Investigators: None

Cooperating Units: Laboratory of Germfree Animal Research, NIAID-112

Man Years (calendar year 1959):

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

To determine the effects of a germfree environment on the relative distribution of various protein components of germfree guinea pigs, mice, and other small laboratory animals. This work is being done to obtain base line values in preparation for contemplated serologic studies on the pathogenesis of parasitic infections in germfree hosts.

Methods Employed:

Serum from animals maintained in germfree tanks on a sterilized ration is compared with that obtained from animals housed in conventional cages and given the same ration. A third group of animals, housed in conventional cages and fed a commercial pellet diet is also studied. Paper electrophoretic studies are carried out according to standard techniques.

Major Findings:

The results of studies on 108 guinea pigs indicate that the

total serum protein content is markedly lowered and that the alpha globulin component is significantly decreased due to the germfree condition. On the other hand, the percentage of gamma globulin does not appear to be altered by the germfree state, i.e. it was no lower than that of conventional animals fed on the special sterilized germfree diet. In both groups, however, lower gamma globulin levels were obtained than were found in conventional animals maintained on the usual laboratory ration.

Experiments with mice indicate that the serum protein picture is not greatly altered by the germfree environment. It was found, however, that the total serum protein level of mice obtained from the regular NIH animal colony was significantly higher than that of conventional mice maintained in a special "clean" environment (free of parasites but not germfree).

Significance to the Program of the Institute:

Before the effects of parasitic infections on the relative distribution of serum protein components of the germfree animal can be studied, it is first necessary to establish baseline values in the axenically reared animal. It is hoped that serum protein studies on animals experiencing for the first time the invasion and development of parasites will shed light on the role played in the defense of the host by circulating antibodies associated with the various globulin components.

Proposed Course of the Project:

The present study is to be extended to determine the effects of age and various nutritional factors on the relative distribution of the serum protein components. Later the response to infection with a variety of parasites will be measured.

Part B included - No

Serial No. NIAID-121-K
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Effects of nutrition on chemotherapy of parasitic diseases

Principal Investigator: William B. DeWitt

Other Investigators: None

Cooperating Units: Laboratory of Parasite Chemotherapy, NIAID-131-D

Man Years (calendar year 1959):

Total:	1 1/4
Professional:	1/4
Other:	1

Project Description:

Objectives:

To determine whether the nutrition of the host can influence the efficacy of parasite chemotherapy.

Methods Employed:

Animals maintained on defined diets either enriched or deficient in certain components are exposed to a single species of parasite and the developing infection is treated either in the prepatent or patent periods. The influence of the diet on treatment is determined by (1) the reduction of the number of parasites, (2) condition of the parasites, (3) growth and survival of the host, and (4) pathologic conditions developing in the host.

Major Findings:

The results of 3 experiments indicate that the efficacy

of stibophen (Fuadin) therapy on mature Schistosoma mansoni infections in mice is greatly enhanced by feeding the mice on an enriched semi-synthetic diet containing 30% animal protein. The worm reduction in mice on the enriched diet was 87%, 95%, and 65% respectively as compared with reductions of 5%, 12%, and 15% in mice on commercial diet.

Significance to the Program of the Institute:

Since parasitic infections occur most frequently in backward areas where malnutrition is also prevalent, it is necessary to determine the relation between the nutritional status of the people and the results obtainable by chemotherapy. Failure of chemotherapy is a common experience in such areas.

Proposed Course of the Project:

The present study is to be extended to determine the specific nutritional factors involved in the enhancement of Fuadin efficacy in schistosomiasis. Other drugs used for the treatment of this and other parasitic diseases will be investigated under similar experimental conditions.

Part B included - No

Serial No. NIAID-121-L
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effects of improving the nutrition of malnourished people infected with Schistosoma mansoni

Principal Investigator: William B. DeWitt

Other Investigators: None

Cooperating Units: Dr. José Oliver-González, School of Medicine, San Juan, Puerto Rico and Dr. Eduardo Medina, Rio Piedras Hospital, Rio Piedras, Puerto Rico.

Man Years (calendar year 1959):

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

To evaluate the effects of providing malnourished people infected with Schistosoma mansoni with an enriched diet containing an abundance of animal protein.

Methods Employed:

Malnourished people suffering from schistosomiasis are given high-protein, high-caloric, vitamin-enriched food over an extended period so that the effects of the improved diet on their well-being and on the parasitic infections may be determined. Such factors as the following are observed before and at various intervals after the patients are given the enriched diet: (1) Nutritional status as determined by physical examinations and biochemical tests, (2) egg-production and viability of eggs of the parasite, (3) antibody levels, (4) skin sensitivity to schistosome antigens, (5) intestinal

uptake of essential nutrients, (6) liver function, as determined by BSP and thymol turbidity measurements and, (7) liver biopsy examinations. After the effects of the improved diet on the health of the patients have been determined, the patients will be given a standard course of treatment so that the effects of the improved nutrition on the efficacy of the drug can be determined. Suitable control patients are likewise to be studied.

Major Findings:

Since this project has just been initiated no conclusions may be drawn at this time.

Significance to the Program of the Institute:

This project is a direct extension of some of our basic laboratory studies which indicate that the normal growth and development of schistosomes is prevented when the experimental host is given a deficient diet. Many of the worms remain sexually immature and are unable to produce eggs, which are important factors in the pathogenic mechanism of schistosomiasis. No information is available concerning the interrelation of diet and schistosomiasis in humans. If, as has been shown in animal experiments, the efficacy of treatment of schistosomiasis in humans can be greatly enhanced by providing an enriched diet, it would be a major accomplishment. As to date no satisfactory treatment of the disease has been discovered.

Proposed Course of the Project:

The project will be pursued along the course indicated above.

Part B included - No

Serial No. NIAID-121-M
1. Parasitic Diseases
2. Helminthic Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Ammonia metabolism and toxicity in relation to liver disease

Principal Investigator: Kenneth S Warren

Other Investigators: None

Cooperating Units: Laboratory of Germfree Animal Research, NIAID-112
Laboratory of Clinical Investigation, NIAID
Postgraduate Medical School, London, England

Man Years (calendar year 1959):

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description:

Objectives:

Study the relation of ammonia toxicity to hepatic coma, the metabolism of ammonia in the bowel, liver and brain, and the passage of ammonia across tissue barriers.

Methods Employed:

Study the effects on ammonia toxicity of oxygen deficiency, various types of liver disease, metabolic inhibitors, and compounds known to cause or aggravate hepatic coma. Investigate the effect of blood pH change on the distribution of ammonia in patients in hepatic coma and the effect on their mental state. Study ammonia production in the bowel by using germfree animals. The specific methods employed are determination of LD_{50}s of ammonia under the above conditions, electrometric pH determinations and ammonia microdiffusion techniques.

Major Findings:

Hypoxia markedly increases the toxicity of ammonia. Many other factors have been studied but have had only a slight effect on ammonia toxicity. Metabolic pH changes cause an acute redistribution of ammonia in patients in hepatic coma, but have no effect on the mental state of these patients. Bacteria are the major cause of ammonia-formation in the bowel.

Significance to the Program of the Institute:

Hypoxia is a frequent concomitant of liver diseases. Ammonia toxicity is generally considered as the primary cause of hepatic coma. Therefore, the use of oxygen as therapy in hepatic coma has been recommended on the basis of the above findings. Work with other factors has indicated that ammonia alone is not the cause of hepatic coma.

Proposed Course of the Project:

Study the effect of ammonia on cerebral oxygen uptake in a larger animal (dog or cat). Study the effect of anoxia and ammonia on cerebral glycolysis in the mouse. Continue investigations into factors that may aggravate ammonia toxicity and study their biochemistry. Examine ammonia production in germfree bowel by in vitro methods.

Part B included - Yes

Serial No. NIAID-121-M

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

Stabenau, J. R., Warren, K. S. and Ball, D. P.: The role of pH gradient in the distribution of ammonia between blood and cerebrospinal fluid, brain and muscle. J. Clin. Invest. 38: 373-383, 1959.

Nathan, D. G. and Warren, K. S: A colorimetric method for the measurement of the brain ammonia of the mouse: the effect of glutamine as the total measurable ammonia. Arch. Biochem. & Biophys., 81: 377-391, 1959.

Warren, K. S and Newton, W. L.: Portal and peripheral blood ammonia concentrations in germfree and conventional guinea pigs. Am. J. Physiol., 197: 717-720, 1959.

Honors and Awards relating to this project:

Dr. Kenneth S Warren received Diploma of Tropical Medicine and Hygiene (England).

Dr. Kenneth S Warren Honorary Research Fellow - Postgraduate Medical School - London, England.

Serial No. NIAID-123-A
1. Parasitic Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
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Part A.

Project Title: Fundamental physiological and biochemical studies on parasites, intermediate hosts, and parasitized animals.

Principal investigator: Theodor von Brand

Other investigators: Teresa I. Mercado, Eleanor J. Tobie, Patricia A. McMahon, Iain B. R. Bowman (Guest Worker, from September, 1959)

Cooperating Units:
1. Section on Steroids, Laboratory of Chemistry, NIAMD.
2. Laboratory of Histology and Pathology, NIDR.
3. Department of Pharmacology, Medical School, Louisiana State University, New Orleans, La.

Man Years (calendar year 1959):

Total:	4
Professional:	2½
Other:	1½

Project Description:

Objectives:

The objective is to gain knowledge on the chemical composition and the metabolism of parasites, intermediate hosts, and parasitized animals.

Methods Employed:

Trypanosomes are cultivated in wholly fluid media and their ability to utilize the non-reducing disaccharide trehalose is studied by the glucose oxidase and related methods. Quantitative determinations are made of the calcareous corpuscles of Taenia taeniaeformis and Cysticercus fasciolaris, the latter being obtained from the Section on Helminthic Diseases. The tissues of the worms are destroyed by alkali and the corpuscles purified by differential centrifugation. Some tapeworms are fixed, sectioned and stained by various techniques to

demonstrate histochemically some of the constituents of the corpuscles. Some of the material is turned over to the Laboratory of Histology and Pathology, NIDR, for collaborative X-ray and electron diffraction studies, as well as electron microscopic studies. The phospholipids of the two tapeworms are isolated and fractionated by a variety of techniques, including differential hydrolysis, chromatography on silicic acid columns, and others; they are analyzed for various constituents by a great variety of different chemical techniques. Freshly isolated liver flukes, specimens starved in axenic surroundings with and without subsequent feeding (obtained from the Department of Pharmacology, LSU) are studied histochemically for glycogen distribution. The unsaponifiable lipid fraction of snails is isolated by a saponification procedure followed by ether extraction and turned over to the Section on Steroids, NIAMD, for isolation and identification of sterols.

Major Findings:

No calcareous corpuscle gradient exists in T. taeniaeformis; the larval form contains twice as much calcareous material as the adult. A considerable amount of calcareous material disappears upon anaerobic incubation of the worms, especially at pH between 5 and 6, less under aerobic incubation or at alkaline pH. The calcareous corpuscles are amorphous, but upon heating with KOH both apatite and brucite crystals are formed. The phospholipids of C. fasciolaris and T. taeniaeformis contain about 88 percent glycerol phosphatide. Serine and ethanolamine determinations indicate that approximately half of the glycerolphosphatide is represented by cephaline. In collaboration with the Section on Steroids, NIAMD, it was shown that the unsaponifiable fraction of T. taeniaeformis contains 98 percent cholesterol, while in Moniezia expansa some 7-ketocholesterol was found besides cholesterol. Starving Fasciola rapidly lose most of the glycogen present in the parenchyma and the muscles, but not from the vitellaria and uterine eggs. These observations are of importance because they can be correlated with physicochemical observations made at the Department of Pharmacology, LSU, of the occurrence of two glycogen modifications in liver flukes.

Significance of the Program to the Institute:

The studies on metabolism and chemical composition of parasites, intermediate hosts, and parasitized animals are essential for an understanding of the pathogenesis of parasitic diseases. They also lay a foundation for a rational approach to chemotherapy.

Proposed Course of the Project:

Investigation of the metabolism of trypanosomes will be continued. The calcareous corpuscle studies will be terminated early next year and attention will be directed towards the anaerobic metabolism of C. fasciolaris. The phospholipid studies will be continued. The Fasciola studies will be continued, but it is expected that they will be terminated next year.

Part B included - Yes

Serial No. NIAID-123-A

PHS-NIH
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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

von Brand, Theodor: Neuere Untersuchungen aus dem Gebiet der Parasitenphysiologie. Ztsch. Tropenmed. Parasitol. 10: 123-134 (1959).

von Brand, Theodor: Neuere Untersuchungen aus dem Gebiet der pathologischen Physiologie parasitischer Infektionen. Ztsch. Tropenmed Parasitol. 10: 135-146 (1959).

von Brand, Theodor, and Tobie, Eleanor Johnson: Observations on the metabolism of the culture form of Trypanosoma congolense. J. Parasitol. 45: 204-208 (1959).

von Brand, Theodor, McMahon, Patricia, Tobie, Eleanor Johnson, Thompson, Malcolm J., and Mosettig, Erich: Chemical composition of the culture form of Trypanosoma cruzi. Exper. Parasitol. 8: 171-181 (1959).

von Brand, Theodor: Der Stoffwechsel der Trypanosomen. Ergeb. Biol. (in press).

von Brand, Theodor: Influencia del tamano, motilidad, ayuno y edad sobre la actividad metabolica. Biologia (Chile) (in press).

von Brand, Theodor: The metabolism of trypanosomes with special reference to Trypanosoma cruzi. Proc. 1 Internat. Congr. Chagas Dis. (in press).

Thompson, Malcolm J., Mosettig, Erich, and von Brand, Theodor: The unsaponifiable lipids of Taenia taeniaeformis and Moniezia sp. Exper. Parasitol. (in press).

Awards: None

Honors: Theodor von Brand was asked to deliver one of the principal lectures before the First International Congress on Chagas Disease, Rio de Janeiro, Brazil, July 1959.

Theodor von Brand was asked to deliver a lecture before the Instituto de Biologia Juan Noe and to preside at a round table conference at the

Institute of Parasitology and also at the Institute of Physiology, Medical School, University of Chile, Santiago, Chile, July 1959.

Theodor von Brand was asked to present four lectures before the Graduate Course in Nematology, North Carolina State College, Raleigh, North Carolina, June 1959.

Serial No. NIAID-123-B
1. Parasitic Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Pathological physiology and histochemistry of parasitic diseases

Principal Investigator: Teresa I. Mercado

Other Investigators: Theodor von Brand

Cooperating Units: None

Man Years (calendar year 1959)

Total:	1 3/4
Professional:	3/4
Other:	1

Project Description:

Objectives:

To gain a better understanding, by means of studies at the cellular level, of the pathologic physiology of parasitic diseases as related to interaction between parasite and host.

Methods Employed:

Various histochemical methods to study the liver glycogen and lipid of normal rats and rats infected with blood parasites (Bartonella muris, Trypanosoma equiperdum, T. equinum, and T. congolense) starved or fed with fructose, untreated or treated with meticortelone.

Organ calcification induced by hytakerol (dihydrotachysterol) or parathyroid hormone in normal rats and rats infected with Plasmodium berghei is studied by means of histochemical methods for the detection of calcium, especially the von Kossa method.

Major Findings:

Liver glycogen synthesis was markedly reduced in animals highly infected with trypanosomes following the administration of fructose and/ or meticortelone; in milder infections the decrease was less significant. The degree and pattern of lipid infiltration, unlike that previously described in malaria (glycogen depletion partially reversed by corticosteroids, and marked centrilobular lipid infiltration) was quite variable, being consistently more pronounced only in T. congolense infections. In bartonellosis the histochemical picture resembled more that of malaria but in addition areas of necrosis occurred that contained glycogen even when the surrounding non-necrotic tissue was glycogen-free. Thus, every infection shows peculiarities with respect to liver glycogen and lipid and such unspecific factors as anoxia and adrenal involvement cannot be the only factors responsible for the liver changes. It is possible that a specific influence originating from the parasite modifies the response of the liver to the unspecific factors. Studies on calcification so far suggest a higher incidence of calcium deposition in rats infected with Plasmodium berghei than in normal rats.

Significance to the Program of the Institute:

Basic cellular studies on the pathologic physiology of parasitic diseases are a necessity in trying to understand the complicated interaction between parasite and host. They may well pave the way for future research directed toward the identification of a specific parasite substance and this would be an invaluable aid for advancement in chemotherapy and immunology.

Proposed Course of the Project:

The present studies on calcification will be continued. Histochemical studies on the liver will be extended to the study of enzyme localization (e.g. alkaline phosphatase, cytochrome oxidase, etc.) during various parasitic infections.

Part B included - Yes

Serial No. NIAID-123-B

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Mercado, Teresa I. and von Brand, Theodor: Histochemical studies of glycogen and lipid in some parasitic infections. J. Inf. Dis., in press.

Honors: None

Awards: None

Serial No. NIAID-123-C
1. Parasitic Diseases
2. Physiology
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Biochemical mechanisms of energy metabolism in normal, aged, and parasitized animals.

Principal Investigator: Eugene C. Weinbach

Other Investigators: None

Cooperating Units: Section of Aging, Laboratory of Psychology, NIMH.

Man Years (calendar year, 1959):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

The objective is to investigate the biochemical changes in energy metabolism associated with biological aging and parasitism.

Methods Employed:

Mammalian tissues are fractionated by mechanical disruption of the cells in appropriate media and the subcellular elements (mitochondria, nuclei, soluble fraction) isolated by differential centrifugation. These cell fractions, isolated simultaneously from normal and parasitized animals, are assessed for their capacity to catalyze exergonic and related reactions of oxidative phosphorylation. Mitochondrial stability is evaluated by detecting alterations in both physical and enzymatic properties. Intramitochondrial substrates and cofactors of metabolism are determined by suitable enzymatic, chemical and spectrophotometric techniques. Radioisotope procedures are used to investigate mechanisms of oxidative phosphorylation and other enzymatic reactions. In collaboration with the Section on Aging, NIMH, similar studies are conducted with tissues from aged rats.

Major Findings:

Isolated liver, brain and kidney mitochondria were found to have the capacity for an appreciable rate of phosphorylation coupled to the oxidation of endogenous substrate. Although the nature of the endogenous substrate is obscure at present, our studies have revealed that its oxidation is linked through pyridine nucleotide, with molecular oxygen as the ultimate oxidant. The stability of isolated liver mitochondria has been shown to be related to the endogenous oxidative phosphorylation. The oxidation of β-hydroxybutyrate to AcP to ATP is impaired in kidney, as well as liver, mitochondria isolated from aged rats. The subsequent oxidation of acetoacetate is not affected by age. Mitochondria isolated from livers of rats parasitized with T. equinum are deficient in their ability to catalyze oxidative phosphorylation. This deficit becomes especially pronounced upon storage of mitochondria. These alterations in biochemical energetics occur only with the same levels of parasitemia at which histochemical changes in the liver tissue were observed previously.

Significance to the Program of the Institute:

This investigation, in addition to its obvious significance to biochemistry in general, contributes to our knowledge of the subtle biochemical changes in energy metabolism associated with parasitism.

Proposed Course of the Project:

During the forthcoming year biochemical studies of senescence temporarily will be discontinued. Efforts will be directed toward a deeper understanding of the basic biochemistry of mitochondria. The studies of biochemical energetics in parasitized animals will be continued.

Part B included - Yes

Serial No. NIAID-123-C

PHS-NIH
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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Weinbach, Eugene C. and Garbus, Joel: Oxidative phosphorylation in mitochondria from aged rats. J. Biol. Chem. 234: 412-417, 1959.

Weinbach, Eugene C.: Stability of oxidative phosphorylation and related reactions in isolated liver mitochondria. J. Biol. Chem. 234: 1580-1586, 1959.

Weinbach, Eugene C. and Garbus, Joel: Coenzyme A content and fatty acid oxidation in liver and kidney mitochondria from aged rats. Gerontologia, in press.

Honors and Awards relating to this project:

Dr. Eugene C. Weinbach was invited to serve as an advisor to WHO Scientific Group on Bilharziasis in Geneva, February, 1959.

Serial No. NIAID-123-D
1. Parasitic Diseases
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biology of trypanosomes.

Principal Investigator: Eleanor J. Tobie

Other Investigators: Theodor von Brand

Cooperating Units: Applied Immunology Section, Laboratory of Immunology, NIAID. Serial No. NIAID-148

Man Years (calendar year 1959):

Total:	1¼
Professional:	3/4
Other:	½

Project Description:

Objectives:

To clarify the life cycle of Trypanosoma rangeli in relation to its vertebrate and invertebrate hosts.

Methods Employed:

Six-day-old rats and Rhodnius prolixus are used as hosts. A colony of R. prolixus is maintained and T. rangeli is maintained in vitro. Rats are infected in two ways, artificially by inoculating culture material IP, and naturally by allowing infected R. prolixus to feed on them. R. prolixus are also infected in two ways, artificially by inoculating culture material or positive fecal material from R. prolixus into the coelomic cavity (CC), and naturally by allowing them to feed on infected rats. Infections in rats are determined by microscopic examination of fresh drops of blood and by in vitro cultivation of blood. Infections in R. prolixus are determined in several ways, by examination of fecal material and hemolymph, feeding on fresh rats, and by dissection and microscopic examination of intestinal contents, hemolymph, and salivary glands. Fresh frozen sections of 5 microns are made of infected R. prolixus, stained and examined microscopically.

Major Findings:

Intestinal infections of R. prolixus can be produced by feeding on infected animals, but these infections have not, as a rule, resulted in salivary gland infections. Salivary gland infections can be produced by inoculating culture material or positive fecal material from R. prolixus into the coelomic cavity.

Flagellates have been localized within the salivary glands of R. prolixus in fresh frozen, stained sections.

Significance of the Program to the Institute:

Biological studies are a prerequisite for subsequent studies.

Proposed Course of the Project:

Studies begun will be continued in order to find out under what conditions the cycle completes itself (from digestive tube to hemolymph) within the insect host, and to localize flagellates in other parts of the insect by studying fresh frozen, stained sections. Studies will be initiated to determine if reproduction of T. rangeli takes place within the vertebrate host or if flagellates inoculated merely sojourn there.

Part B included - No

Serial No. NIAID-127-4
1. Parasitic Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on toxoplasmosis

Principal Investigator: Leon Jacobs

Other Investigators: Milford N. Lunde, Marjorie L. Melton, Anastasia S. Stanley, and Jack S. Remington (to June, 1959)

Cooperating Units: Ophthalmology Branch, NINDB

Man Years (calendar year 1959)

Total:	5½
Professional:	3½
Other:	2

Project Description:

Objectives:

The accumulation of data on the occurrence of toxoplasmosis as an acute and chronic infection of man and animals; the study of the biology of the proliferative parasite and the cyst, with the aim of evaluating their importance in the epidemiology of toxoplasmosis and in the production of chronic disease; the determination of the usefulness of immunological tests in diagnosis, and the identification of antigens and antibodies; the description of factors related to the virulence of Toxoplasma, and of those involved in susceptibility and immunity of the host, and in the occurrence of congenital disease; the elucidation of the mechanism of cyst-formation, relative to factors in the host; the critical study of toxoplasmicidal agents against proliferative parasites and cysts; the study of the role of hypersensitivity in ocular toxoplasmosis.

Methods Employed:

Standard procedures of animal inoculation plus special techniques for injection into the anterior or posterior chambers of

the eye. A microisolation apparatus is used for handling cysts. Histochemical procedures are being initiated to complement electron-microscopic work being done in collaboration with NINDB. Tissue cultures are used for morphological, histochemical, and electron-microscopic studies. The dye and hemagglutination tests are used for serological studies, and the antigens are investigated by biochemical and electrophoretic techniques.

Major Findings:

The cyst wall of Toxoplasma has been found to be a complicated structure rather than a simple membrane. In brain sections studied by the electron microscope, the wall appears to be continuous with the surrounding brain substance; in isolated cysts the wall has the same appearance although it seems to be a separate membrane when viewed with the light microscope. The cyst swells in saline, and the encysted organisms rapidly lose glycogen when stored in saline.

The ability of encysted Toxoplasma to withstand peptic digestion, albeit for only a relatively brief period, allows us to distinguish between encysted and proliferative forms in progressing infections.

By the digestion technique, we have been able to discover 3 additional cases of residual infection in human uteri removed at hysterectomy. The most significant thing about this finding is that in all of these cases the dye test titers were low, 1:16 to 1:64, comparable with antibody levels found in the general population. Thus, encysted parasites can persist ~~outside~~ in the extraneural viscera without stimulating high levels of antibody-production.

The conditions under which congenital transmission of Toxoplasma occurs in animals with acute and chronic infections have been studied more exactly. Contrary to previous reports, it has been found that Toxoplasma can invade the foetus without prior proliferation in the syncytial trophoblast. The occurrence of congenital transmission from chronically infected mothers has occurred most frequently with strains of the parasite which produce late parasitemias.

Occasional spontaneous parasitemias have been found in mice, rabbits, and guinea pigs infected with various cyst-producing strains of Toxoplasma. Rats with chronic infections with these strains do not show parasitemias; cortisonized rats occasionally show parasitemia. The injection of hyperimmune serum into mice with chronic infections results in some increase in the number showing parasitemia.

The hemagglutination test has been evaluated on a variety of experimental animals infected with virulent and avirulent Toxoplasma strains and on serum specimens of domestic animals submitted from various sources. In monkeys and rabbits, infected with the RH strain, the HA test became positive 4 - 5 days after the dye test. In the monkey there was evidence of an early antigenic phase, as indicated by hemagglutination-inhibition, prior to the appearance of antibodies. In rats, the HA test becomes positive 2 to 3 weeks after the dye test, or even as late as 10 weeks when the infection is produced by an avirulent strain. On the other hand, pigeons showed HA antibodies 1 or 2 days prior to the dye test regardless of the parasite strain. In naturally infected dogs, the HA test is not as sensitive as the dye test. In naturally infected sheep, the HA test agrees very well with the dye test.

Various fractions of the hemagglutinating antigen have been prepared. One of these, in contrast to the whole antigen, appears to be stable on storage. Another fraction produces hemagglutinating antibodies in rabbits without detectable dye test antibodies.

In tissue culture studies, a relatively avirulent strain of Toxoplasma has persisted in culture without obvious cytopathic effect. This strain is also apparently less susceptible *in vitro* to the action of pyrimethamine than a rapidly multiplying strain.

Significance to the Program of the Institute:

Knowledge of the transmission of Toxoplasma in nature is requisite as a basis for recommendations for prevention of the infection. Continued work is necessary to explain mechanisms of transmission to herbivores and vegetarian human beings.

The analysis of antigens derived from Toxoplasma has importance in relation to an understanding of the course of the disease and the action of various antibodies, as well as diagnosis. Continued work on the hemagglutination test may establish it as the best practicable procedure for diagnosis of human toxoplasmosis.

The factors which are involved in alterations in host-parasite balance in chronic Toxoplasma infections, formation and rupture of cysts, invasiveness and pathogenesis of strains, and host-parasite relations at the cellular level, are all subjects for basic study. Investigation of these problems can be expected to furnish explanations for clinical observations and to contribute to a general understanding of chronic infections. This is of great importance in relation to ocular disease and may also reveal instances of chronicity in systemic infections.

Proposed Course of the Project:

Attention will be paid to: the epidemiology of toxoplasmosis in herbivorous animals; the circumstances of cyst-formation and rupture in chronic infections; the relation of cysts and dormant parasites to chronic disease; the relation of antibodies to particular antigenic components and to persistence of chronic infections; the effect of drugs on cysts and on activity of the parasite during chronicity; and the relation of local tissue immunity to the spread of challenge infections.

Part B included - Yes

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Lunde, M. N. and Jacobs, L.: Characteristics of the Toxoplasma hemagglutination test antigen. J. Immunol. 82: 146-150, 1959.

Kaufman, H. E., Melton, M. L., Remington, J. S., and Jacobs, L.: Strain differences of Toxoplasma gondii. J. Parasitol. 45: 189-190, 1959.

Jones, Frances E., Melton, Marjorie L., Lunde, Milford N., Eyles, Don E., and Jacobs, Leon: Experimental toxoplasmosis in chickens. J. Parasitol. 45: 31-37, 1959.

Frenkel, Jack K. and Jacobs, Leon: Ocular toxoplasmosis: Pathogenesis, diagnosis, and treatment. Arch. Ophth. 59: 260-279, 1958. (omitted from 1958 report).

Jacobs, Leon and Wiczer, Donald W.: Congenital toxoplasmosis. Clin. Proc. Children's Hospital, Washington, D. C. 15: 212-218, 1959.

Kaufman, Herbert E., Remington, Jack S., Melton, Marjorie L., and Jacobs, Leon: Relative resistance of slow-growing strains of Toxoplasma gondii to pyrimethamine (Daraprim). Arch. Ophth. 62: 611-615, 1959.

Jacobs, Leon, Remington, Jack S., and Melton, Marjorie L.: The resistance of the encysted form of Toxoplasma gondii. J. Parasitol. (in press).

Jacobs, Leon, Remington, Jack S., and Melton, Marjorie L.: A survey of meat samples from swine, cattle, and sheep for the presence of encysted Toxoplasma. J. Parasitol. (in press).

Jacobs, Leon: Experimental studies relating to ocular infection in toxoplasmosis. Acta Ophthalmologica (in press).

Honors:

Dr. Leon Jacobs was appointed to the faculty of the George Washington School of Medicine as Special Lecturer in Medical Parasitology.

Dr. Leon Jacobs was invited to participate in a special conference on the diagnosis of toxoplasmosis, sponsored by the Government of Denmark, August, 1959.

Dr. Leon Jacobs was appointed:

Chairman, Committee on Business Operations, American Society of Parasitologists

Chairman, Committee on Cooperative Publication, Conference of Biological Editors

Representative to National Research Council, American Society of Tropical Medicine and Hygiene

Dr. Leon Jacobs was awar ed a Fulbright fellowship for 1960, for research on ovine toxoplasmosis in New Zealand.

Serial No. NIAMD-12I-B
1. Parasitic Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Entamoeba histolytica and other parasitic protozoa

Principal Investigator: Louis S. Diamond

Other Investigators: None

Cooperating Units: Laboratory of Veterinary Biochemistry, Walter Reed Army Institute for Research, Mr. Nathaniel Rothstein.

Biophysics Laboratory, Naval Institute for Medical Research, Dr. Harry T. Meryman and Mr. Emmanuel Kafig.

Man Years (calendar year 1959):

Total:	1 3/4
Professional:	3/4
Other:	1 3/4

Project Description:

Objectives:

1. Entamoeba histolytica and amoebiasis

The long range objectives of investigations on E. histolytica and amoebiasis remain the same as for the previous year and are concerned with the metabolism of the parasite, methods of diagnosis, mechanism of pathogenesis, factors of host susceptibility and differences in strain virulence.

Since the experimental solution of many unsolved problems in amoebiasis are dependent upon the availability of large supplies of E. histolytica cultured without associated microorganisms, development of such a culture technique is given top priority. Meanwhile,

until this goal is attained, attempts to solve some of the problems are being made with the use of cultures of E. histolytica grown in association with single species of microorganisms.

Similar investigations are carried out on Entamoeba spp. of lower animals to aid in understanding the biology of the amoebae and possibly in the solution of specific problems concerning E. histolytica and human amoebiasis.

To cultivate axenically Trichomonas tenax, the oral trichomonad of man.

The ability to preserve protozoan cultures for indefinite periods would free the technical staff from burdensome, time-consuming maintenance work. Additionally it would serve to preserve the original characteristics of freshly isolated strains. The objectives of this phase of the work are to devise techniques for preserving protozoan cultures by freezing and storage at low temperatures and by freezing and storage in the dry state.

Methods Employed:

Development of or improvement of techniques for: axenic cultivation of Entamoeba species in macrocultures, cultivation of E. histolytica with single species of microorganisms in various media, cyst production in vitro, by manipulation of culture media; use of dark field microscopy and fluorochrome stains to study cytochemical components of amoebae; production of antigens from harvests of cultures and extractions of the harvests.

Strains of T. tenax obtained directly from the patient or indirectly after passage through multifloral cultures are first freed of associated microorganisms with the aid of antibiotics and then introduced into media made up into a variety of physical forms and containing various tissue extracts and growth promoting substances.

Protozoa obtained from cultures are suspended in glycerol or other suitable agents and brought slowly to temperatures of -25° or -35° C, then stored at these temperatures or at temperatures of -79° or -197° C.; or the protozoa are frozen rapidly by plunging them into an environment of frozen CO_2 at -79° and stored in liquid N at -197°; or protozoa are placed in a vacuum where they are supercooled, frozen and finally dried.

Major Findings:

Limited trials indicate the possibility of replacing the living cells ordinarily required for maintenance of E. histolytica in vitro with extracts of mammalian embryo. Emphasis is being placed on the development of macroculture techniques rather than on microcultures. Previous research has demonstrated in the latter certain inherent characteristics which lead to uncontrollable variation.

Antigens of E. histolytica are now in the process of preparation. Heretofore cultures of E. histolytica grown in association with a single bacterial species have been employed as a source of antigenic material. The antigens employed in the present work are being prepared from cultures of E. histolytica grown in association with Trypanosoma cruzi. This type of culture was chosen primarily because the freedom of the medium from insoluble nutrients obviates absorption of amoebic antigens on solid particles.

A vital fluorochrome stain technique devised by Diamond and Rothstein has been used for studying the distribution of DNA and RNA in amoebae. DNA is seen in the endosome.

Cysts produced from starch-containing cultures are at present being used for establishment of E. histolytica - T. cruzi cultures. These cultures are in turn employed in this laboratory as a starting point for axenic cultivation work and for germ-free experiments conducted by the Laboratory of Germ-free Animal Research.

Cultural studies have been carried out on two parasitic amoebae of reptiles; one of these, E. invadens, produces a highly fatal amebiasis in snakes which pathologically resembles that produced by E. histolytica in man, the other, E. terrapinae, is a commensal of turtles. The present investigations have led to the development of a technique for the axenic cultivation of E. terrapinae and an improved method of cultivating E. invadens axenically.

T. tenax when first introduced into media designed for axenic cultivation grows luxuriantly, but it has not been possible to maintain this type of growth past the first transfer and death of the culture has always ensued prior to the sixth transfer.

Incidental to investigations of trichomonads of man, a monocercomonad isolated in axenic culture from the gall bladder of an infected snake has been identified as a new species. This parasite is of particular interest to the systematist because it belongs to a genus representing the most primitive trichomonad flagellates. Application of the vital stain-darkfield technique to the study of the morphology of the parasite led to the demonstration by differential staining of the parabasal body of a living trichomonad.

This structure is considered to be of great significance in the metabolic economy of trichomonads and as a phylogenetic marker of the group.

Axenically grown E. invadens suspended in glycerol and brought slowly to -25° C. will survive for 2P days when stored at this temperature but fails to survive when stored at -79° C.

Experiments carried out in cooperation with Dr. Meryman and Mr. Kafig of the Naval Medical Research Institute have demonstrated that:

1) axenically grown E. invadens, E. terrapinae and the monocercomonad can be frozen rapidly at -79° then stored at -197°.

2) the same species can be dried, then reconstituted in fresh medium.

Because provisions for the safe handling of human pathogens were not in operation in the laboratory where these experiments were performed, only parasites known to be non-pathogenic for man were employed. However, there is no reason to doubt that similar results can be obtained with such parasites as E. histolytica, T. vaginalis and T. cruzi.

Significance to the Program of the Institute:

As has been pointed out in the previous year, the techniques developed for the axenic cultivation of parasitic protozoa may find application in studies not only of other parasites but mammalian cells as well. Knowledge gained in the development of cryogenic methods for the preservation of protozoa will undoubtedly be of use in developing similar techniques for preservation of other types of cells. The vital stain-darkfield technique devised for the morphological and cytochemical study of parasitic protozoa may be used for study of tissue culture cells and tissue culture cells infected with a variety of disease agents. Because of the ease with which the monocercomonad can be grown in axenic culture and the luxuriousness of the growth this parasite should serve as an excellent tool for the comparative biochemist.

Proposed Course of the Project:

The following efforts will be made: Continuation of attempts to grow E. histolytica and T. tenax axenically; production of antigens of E. histolytica and development of improved diagnostic tests; study in experimental animals of the immune phenomena in the intestinal tract and hypersensitivity in amoebic hepatitis; evaluation of factors associated with pathogenicity; refinement of

techniques for the preservation of protozoa by freezing and storage at low temperatures and in the dry state.

Part B included - Yes

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Diamond, L. S.: The axenic cultivation of two reptilian parasites, Entamoeba terrapinae Sanders and Cleveland, 1930 and Entamoeba invadens Rodhain, 1934. J. Parasitol. (in press)

Honors and Awards relating to this project: None

Serial No. NIAID-NIH-4
1. Parasitic Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calender Year 1959

Part A.

Project Title: Trichomoniasis

Principal Investigator: Lucy V. Reardon

Other Investigators: Louis S. Diamond

Cooperating Units: Laboratory of Clinical Investigations, NIAMD
Serial No. NIAID-20-(C)
Laboratory of Germfree Animal Research, NIAID
Serial No. NIAID-115
Laboratory of Pathology and Histochemistry, NIAMD
Clinical Investigations Branch, NIDR

Man Years (calendar year 1959):

Total:	2½
Professional:	1½
Other:	1

Project Description:

Objectives:

The study of the pathogenesis of Trichomonas vaginalis, especially in regard to differences in strains of the parasite; transformation of virulence of strains; freezing as a means of maintaining strain characteristics; antibody-formation and hypersensitivity in relation to resistance and pathogenesis; the action of purported trichomonicides, as determined in vitro and in vivo, especially in respect to their evaluation as systemic therapeutic agents; the possible relation of oral protozoa, T. tenax and Entamoeba gingivalis to peridontal disease.

Methods Employed:

Pathogenesis of strains of T. vaginalis is tested in various strains of mice and germfree guinea pigs. Criteria for strain differences are also sought by in vitro studies of the organism

with special reference to gas-production. Antigens prepared from cultures by various methods and antisera obtained from experimentally infected or immunized animals are tested in the hemagglutination test. Evaluation of purported trichomonicidal agents is made by culture techniques and in vivo in mice experimentally infected with the virulent C-1 strain of T. vaginalis. For studies on transformation of virulence in Trichomonas strains, organisms of the non-virulent R strain are associated with homogenates containing DNA of the virulent C-1 strain. Alterations are determined by measuring the virulence in mice. Freezing strains of T. vaginalis for storage is carried out by rapid freezing in dry ice and by slow freezing to -25°C in apparatus designed to control cooling at the rate of one degree per minute. Diseased or normal tissues from patients with periodontal disease, obtained following surgery (collaboration with the Clinical Investigations Branch, NIDR), are examined for oral protozoa, T. tenax and E. gingivalis by direct examination of the material and by examination of cultures.

Major Findings:

Studies on the infectivity and pathogenicity of T. vaginalis consisted largely in retesting strains of the trichomonads for virulence and of mouse strains for susceptibility to T. vaginalis. The retests confirmed earlier studies which noted that a wide range of virulence occurred among trichomonad strains and that correlation between virulence of strains in mice and pathology in the original host was not well established. Retests of mouse strains likewise confirmed previous data that no strain was susceptible to R organisms and all were susceptible to C-1 organisms. In collaboration with the Laboratory of Pathology and Histochemistry, studies on the histopathology of the liver in mice infected with the virulent C-1 parasite were continued.

In collaboration with the Laboratory of Germfree Animal Research, NIAID, additional experiments were made using the C-1 strain of T. vaginalis. Concentrates of the organism were injected subcutaneously into germfree guinea pigs, mono-contaminated guinea pigs (guinea pigs previously infected with a single species of bacterium, in this case an unidentified micrococcus) and in conventional pigs. With the germfree animals, as in previous experiments, large palpable swellingsor abscesses occurred at the site of injection and were distended with gas; with the mono-contaminated animals the swellings were less prominent or did not occur as is the case with the conventional pigs. Starvation, a ketogenic carbohydrate diet, and dosage with Alloxan, failed to alter the course of experimental infection in mice.

Studies on gas production *in vitro*, as a criterion for strain differentiation in *T. vaginalis* and possible correlation with virulence, were initiated with comparative experiments of the non-virulent R and the virulent C-1 strain, in 2 media: The NIH medium (in current use in this Laboratory for the cultivation of *T. vaginalis*) and in the medium of Diamond, 1957, with and without agar. Evolution of gas was observed only in the Diamond medium with agar and only with the non-pathogenic R strain. The amount of gas produced was too small for analysis.

Sera of guinea pigs immunized by intraperitoneal injection of washed concentrates of trichomonads and titrated with antigens prepared from distilled water lysates of *T. vaginalis* gave high titers in the hemagglutination test. Higher titers were found in the sera of pigs immunized with the virulent C-1 strain than with sera of pigs immunized with the non-virulent R strain. Guinea pigs were also immunized with *Trichomonas* enzymes derived from concentrates of the parasite. The antisera, when tested with the enzyme material for antigen, gave high titers in the hemagglutination test.

Three antibiotics furnished by the Food and Drug Administration, Division of Antibiotics, were tested *in vitro* for trichomonicidal activity. The 3 drugs were: Neomycin 397, NRF; Candidicin NRF 405-10; and RS-2712. Candidicin NRF 405-10, and RS-2712, at concentrations as low as 1 micrograms per ml of medium, inhibited growth of *T. vaginalis*. Neomycin did not inhibit growth of the organisms. Streptovitacin A (Upjohn) effected complete kill of *T. vaginalis* at 30 micrograms per ml and inhibition at 2 micrograms/ml. Candidicin given orally at 1 mg/kg did not protect mice from infection with *T. vaginalis*. Streptovitacin has been found too toxic for tests in mice.

Experiments were instituted with a view to transforming the non-virulent R strain by association with homogenates containing DNA of the virulent C-1 strain. The homogenates were prepared by sonication of concentrates of organisms from cultures. R organisms in various concentrations were exposed to the homogenates and then tested either directly in mice or after cultivation. Thus far no transformations have been achieved, but the techniques have been perfected so that further studies are now possible. When rapidly frozen and stored in dry ice, survival of the R strain was obtained for up to 3 weeks.

In collaboration with the Clinical Investigations Branch, NIDR 18 patients with periodontal disease have been studied for information on the possible bearing of *T. tenax* and *E. gingivalis* on the disease. Examination of diseased tissue from 15 patients showed that 8 were positive for *E. gingivalis* only, 2 for *T. tenax* only,

3 for both organisms, and 2 negative. Normal tissue from 2 of the above patients was positive respectively for E. gingivalis and T. tenax only, agreeing with findings from the counterpart diseased tissues. Normal tissues from 3 other patients with the disease were negative.

Significance to the Program of the Institute:

Not changed from calendar year 1958 except: Transformation of virulence in strains of Trichomonas would furnish evidence of genetic transfer of characters and constitute a basic contribution in this field; conclusive data that oral protozoa, E. gingivalis and T. tenax either do or do not play a role in periodontal disease would settle a question of long standing; a finding that the organisms do play a part in periodontitis would have an important bearing on control of the disease.

Proposed course of Project:

Continued work is contemplated on the usefulness of the hemagglutination test for the diagnosis of trichomoniasis, using serum and vaginal exudates as possible sources of antibody, depending on the availability of specimens from LCI, NIAID. Efforts will be made to relate various aspects of the metabolic or cultural characteristics of T. vaginalis strains to their pathogenicity. Studies on the transformation of virulence of strains of the parasite will be continued. The collaborative project with NIDR will be continued until sufficient data are obtained.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Newton, W. L., Reardon, L. V., and DeLeva, A. M.: A comparative study of the subcutaneous inoculation of germfree and conventional guinea pigs with two strains of Trichomonas vaginalis. Am. J. Trop. Med. and Hyg. (in press).

Honors and Awards relating to this project: None

Serial No. NIAMD-IZT-D
1. Parasitic Diseases
2. Protozoal Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemistry of Trichomonas vaginalis

Principal Investigator: Harry D. Baernstein

Other Investigators: Lucy V. Reardon

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1
Professional:	1
Other:	0

Project description:

Objectives:

To study Trichomonas vaginalis in several of its biochemical aspects: cultivation, intracellular enzymes, and relations with its host.

Methods Employed:

Weekly harvests of 4-6 grams wet weight of organisms harvested from pure cultures are homogenized in a sonic oscillator and the harvests fractionated using specific assay mixtures as a guide to the isolation of individual enzyme activities. Some studies are made of the rate of growth, size of inoculum, incubation time, composition of medium in order to get maximum yields of active organisms. Spectrophotometric studies are employed to evaluate the quality of the harvest and an electronic counter used to study the rate of cell rupture.

Major Findings:

Improvements in the medium for cultivating T. vaginalis have

doubled the yield and greatly improved the activity of extracts. A study of the size distribution of particles in the Coulter counter has shown that the organisms are quickly broken in the 9KC sonic oscillator giving rise to many particles about 1 micron in diameter. These particles are further broken up into smaller particles as sonication is prolonged. Malic dehydrogenase, which in other organisms appears particle-bound, appears in these sonicates as a cytoplasmic enzyme, probably due to the rupture of the small particles.

The properties of a partially purified malic dehydrogenase have been determined and the results prepared for publication. Attempts have thus far failed to find enzymes of the tricarboxylic acid cycle, other than lactic and malic dehydrogenases, which may or may not be functioning in the cycle. Phosphotransacetylase could not be demonstrated because of the presence of a phosphatase which split acetylphosphate, a substrate for coenzyme A formation. Fumarase, aconitase, and isocitric dehydrogenase, could not be demonstrated. However, further work is necessary before the absence of these enzymes can be definitely stated.

Significance to the Program of the Institute:

Establishing pathways of metabolism of T. vaginalis will contribute to understanding the general problem of anaerobiosis, which is relatively undeveloped. The question of the presence or absence of the tricarboxylic acid cycle in anaerobes is uncertain, owing to lack of information regarding pathways for reoxidation of reduced pyridine nucleotides. Our work has shown the presence of three systems contributing to reoxidation of reduced DPN, and Wintschafter has shown a fourth system. However, nothing short of demonstrating other enzymes of the cycle will suffice. The failures thus far may be due to homogenization procedures or the presence of other enzymes. In the absence of a complete Krebs cycle, the elucidation of other mechanisms for oxidizing DPNH should be of general biochemical interest.

Studies of the metabolism of T. vaginalis may aid us eventually in understanding strain differences of the parasite. Furthermore, some of our purified fractions of T. vaginalis may be studied as antigens and transforming agents.

Proposed Course of Project.

Further studies of the enzymes of T. vaginalis are proposed. In addition to tricarboxylic acid cycle pathways, the problem of pentose synthesis, coenzyme A reactions, and hydrogen and carbon dioxide production are proposed.

Our present medium is adequate but unnecessarily complicated and expensive. It is hoped that some improvements in this direction can be made.

Purified enzyme solutions can be used as antigens and immune sera tested for specific antienzymes.

In collaboration with other members of the section preparations can be tested for transforming reactions related to pathogenicity of certain strains of T. vaginalis.

Part B included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications.

Publications other than abstracts from this project:

Baernstein, H. D.: Lactic dehydrogenase in Trichomonas vaginalis. J. Parasitol. 45: 491-498, 1959.

1. Zoonosis Division
2. Protozoan Diseases
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Studies on Pneumocystis and Sarcocystis

Principal Investigator: Leon Jacobs

Other Investigators: Marjorie L. Melton and Milford N. Lando

Cooperating Units: Laboratory of Pathology, NIAMD

Man Years (calendar year 1959)

Total:	½
Professional:	½
Other:	0

Project Description:

Objectives:

To develop methods for handling Pneumocystis and Sarcocystis in experimental systems, to clarify the nature of these organisms, determine their pathogenesis, and devise immunological procedures for recognizing infection with them.

Methods Employed:

Pneumocystis - containing tissues obtained from experimental animals or human cases are examined microscopically by impression smears and sections stained with a variety of reagents. Attempts are made to cultivate the organism from positive tissues by inoculation into chick embryos, tissue cultures, and various culture media.

Sarcocystis is harvested from muscle of various animals and studied for the presence of a toxin. The studies are based on a modification of the dye test for toxoplasmosis, with the sera of infected animals, to see if a test for sarcosporidiosis can be developed. Extracts of spores are used in the preparation of antigens for a hemagglutination test. Experimental transmission of infection to animals is attempted with the spores.

Major Findings:

Yeast-like organisms were observed in tissue cultures and bacteriological media inoculated with lung homogenates from rats showing spontaneous pneumocystosis after prolonged cortisone treatment. Some methenamine-silver preparations of lung were consistent with this finding.

A technique was developed for harvesting large quantities of Sarcocystis spores from infected muscle. The technique involves simply digestion with 1% trypsin and passage of the digest through gauze. Preparations of lysed spores exhibit a high toxicity for rabbits inoculated intramuscularly.

Significance to the Program of the Institute:

Pneumocystosis has been described as a highly fatal epidemic disease of infants in central and northern Europe. Occasional adult cases have also been described, a few in this country. The organism has been described by various workers as a protozoan, by others as a fungus. It is of utmost importance to develop methods of handling it in the laboratory so that studies can be done on its identification and on establishing its role in disease-production.

Sarcocystis is a very common parasite of meat animals as well as of various other mammals, including man, and of birds. It appears structurally to bear some resemblance to Toxoplasma and therefore is of biological interest and may furnish some clues as to transmission. Because of the powerful toxin in sarcospores and because human beings are always being exposed to this toxin and to the spores, in the meat they consume, it appears necessary to characterize the toxin and to explore the possibility that Sarcocystis may be the cause of some human disease.

Proposed Course of the Project:

Further efforts will be made to cultivate Pneumocystis from infected tissue, to make certain that the yeast-like organism thus far found is not a contaminant. Attempts will then be made to reproduce the disease in conventional and in germfree animals, utilizing the organism from culture.

The toxin of Sarcocystis will be characterized as to its composition, stability, etc. Attempts will be made to study the transmission of Sarcocystis in laboratory animals, and to develop serological tests for the infection.

Part B included - No

Laboratory of Parasite Chemotherapy, NIAID

Summary statement of research activities, calendar year 1959

This country's commitment to the world-wide program of malaria eradication and the long-term interest in malaria by most of the senior staff contributed to a research effort, during the past year, largely directed toward problems identified with that disease. Special emphasis was given to malaria chemotherapy as applied to eradication. Inasmuch as our regular facilities provide for only a minimum of clinical material, which continues to become less each year, facilities at the Federal Penitentiary at Atlanta, Georgia were again made available. Studies have been in progress there since April.

The Program of the Section on Epidemiology has included studies on virus-parasite associations but lacked an investigator equipped to study factors controlling the ability of insect vectors to transmit viruses, protozoa, and other disease-producing organisms. The addition of a new member to the staff has permitted the inauguration of studies involving insect tissue culture and acquisition of disease-producing agents by insect larvae. Another personnel change has established studies on the mechanism of drug action; the long-term effort to be directed toward intestinal parasites of man.

The program of the Section on Chemotherapy was strengthened by the addition of an established pharmacologist with auxiliary interest and training in parasitology and entomology.

MALARIA - HUMAN Single doses of chloroquine (Aralen) as low as 75 mg. (base) per week will result in complete suppression of Chesson strain vivax malaria even under conditions of repeated infection via mosquito bite.

Primaquine given as a single 30 mg. dose, weekly, or as a single 3.0 mg. dose, daily, beginning the day before infection by mosquito bite, did not prevent or suppress infection. The drug is sporontocidal at a dosage as low as 1.5 mg., single dose, daily, against either Plasmodium vivax or P. falciparum.

Preliminary results against Chesson strain vivax suggest that the "Russian drug" quinocide, an 8-aminoquinoline closely allied to primaquine, has no advantages over primaquine and may be more toxic.

A strain of P. vivax obtained from the State of Merida in eastern Venezuela, where the population has been under mass suppression with pyrimethamine, was found to have a prepatent period of 13 to 17 days; the incubation period was also 13 to 17 days (data from 10 patients). The parasites of this strain were found to be resistant to a dose of 50 mg. and of 100 mg. of pyrimethamine. This was true whether the infection was induced by blood inoculation or by sporozoites.

MALARIA - MAMMALIAN Morphological studies of two strains of P. cynomolgi have been made which will be useful as guidelines in chemotherapy studies and in studies on in vitro growth. Growth has been seen from the fourth through the ninth day and mature parasites have been observed as late as the 120th day. Reaction of the host was lacking in most monkeys, but was found in one animal. Size changes through the cycle have been studied, and it was found that on a given day the size was consistent. Distribution of parasites was found to be uniform throughout the liver and fit the Poisson distribution well.

Cell lines have been established from monkey liver and other sources. Considerable success has been achieved in maintaining organized liver tissue in vitro. Good survival of organized fragments of adult monkey tissue has been obtained for as long as 10 days which is sufficient time for P. cynomolgi and other malaria parasites to complete an exoerythrocytic cycle. The ability to maintain liver is a major step and is basic to studies of the parasites in vitro.

Exoerythrocytic stages of P. cynomolgi have been grown in vitro for two and four days from fragments of tissue taken from heavily infected monkeys. The parasite increased greatly in size during the period of in vitro maintenance. The cytology of the parasites compares well with normal in vivo parasites. This may represent the first in vitro cultivation of mammalian exoerythrocytic parasites.

A monkey was inoculated with cultured material taken from an infected monkey at seven days after sporozoite inoculation, then maintained in culture for two additional days. The monkey developed an infection.

Antibodies to a sporozoite antigen of P. gallinaceum have been produced in rabbits. This affords a tool which may be useful for in vitro as well as in vivo studies. After conjugation with fluorescein isothiocynate blood forms were stained with apparent success. These preliminary results indicate that this technique may be useful for detecting malaria parasites. The problem is of interest also because the antigenicity of sporozoites has been questioned.

Monkeys treated with pyrimethamine prior to infection did not develop detectable exoerythrocytic parasites in the liver. Since parasitemia occurred eventually, in some of the monkeys, parasites must have survived at sub-patent levels, survived in a form difficult to detect, or survived in tissues other than the liver.

When pyrimethamine treatment was initiated on the sixth day after injection of sporozoites, an effect was evident within 24 hours and

marked after 48 hours. Subsequent to 48 hours, the parasites became vacuolated and disorganized. They showed signs of swelling and by 120 hours after treatment were so altered as to be hardly distinguishable. Very little cellular reaction developed, although occasionally mononuclear cells accumulated around the dying parasites.

When primaquine was given prior to infection, no parasites could be found in the livers. When primaquine was initiated on the sixth day of treatment, an effect was not discernable for 72 to 96 hours after treatment. At 120 hours, after treatment, many parasites appeared as dead, amorphous, dark-staining, shrunken masses without cellular reaction. Studies are now in progress to follow the 'dying' parasites for longer periods.

Both primaquine and pyrimethamine retarded, then stopped the growth of the parasites. This resulted in keeping parasites in the liver at a time the majority would have ruptured and initiated blood infections.

DRUGS - MECHANISM OF ACTION The results using P. gallinaceum indicate that the drugs fall into groups on the basis of their mode of action. Thus, pyrimethamine and chloroguanide specifically inhibit DNA synthesis, the former under all conditions studied, and the latter only in vivo. The drug effect is reversible only by thymidine, indicating that the drug inhibits the enzymatic conversion of deoxyuridine to thymidine.

Drugs with a quinoline or acridine nucleus (quinine, chloroquine, quinacrine) inhibit respiration, RNA, and DNA metabolism apparently nonspecifically through interference with energy conservation by the parasite. Studies with quinine in greater detail revealed that the drug action is dependent on a high potassium ion concentration surrounding the parasite, such as occurs inside the red cell. Thus, the action of quinine and related drugs on the malarial parasite and heart muscle may be similarly connected with potassium ion metabolism or transport.

In studies involving Pseudomonas sp., pyrimethamine inhibited growth, glucose utilization, and pyruvate utilization to approximately the same extent. At the levels tested, formate utilization was not inhibited. Of many substances tested, pyruvate itself was the only material capable of reversing pyrimethamine inhibition.

Preliminary experiments have shown that in the case of P. gallinaceum, the DNA content of infected cells appears to be no different from that of uninfected cells. On the other hand, the RNA content of infected red cells is five times that of uninfected chicken erythrocytes.

DRUGS - PHARMACOLOGY AND LACHRSIOLOGY (FATE)

An *in vitro* system for chloroquine metabolism was found in rabbit liver homogenates. Chloroquine was metabolized at a rate of about 45 per cent per hour per gram of tissue at pH 7.4 in an atmosphere of 95 per cent oxygen. In the presence of SKF 525-A, Lilly 18947, and pyrimethamine at 1×10^{-4}M, chloroquine metabolism was inhibited 80, 90, and 100 per cent, respectively. The metabolic activity of liver homogenate was virtually lost after dialysis but could be restored by the addition of a TPN-H generating system. One of the metabolic products is acetaldehyde and evidence suggests that both alkyl groups of the parent compound are oxidized *in vitro*.

Administration of metabolic inhibitors to rabbits in combination with chloroquine gave rise to higher blood levels and a prolongation of the half-life of the antimalarial drug. However, in most cases, metabolic inhibition beyond 48 hours could not be achieved because of the rapid turnover of the prolonging agent.

Chloroquine was administered orally in combination with a metabolic inhibitor (SKF-525-A, Lilly 18947, pyrimethamine and isoniazid) to mice infected with *P. berghei*. After a single dose of chloroquine in combination with any one of the various inhibitors, the appearance of parasites in the blood was suppressed for a longer time than with chloroquine alone. The chloroquine-SKF combination at 5 mg/kg had four times the suppressive action of chloroquine alone. The mechanism of action in all cases was inhibition of chloroquine metabolism. None of the metabolic inhibitors employed, except pyrimethamine, had any antimalarial effect. On the contrary, some stimulated parasite development.

The physiological disposition of radioactive pyrimethamine in the rabbit after oral and parenteral administration at a dose of 0.5 mg/kg was determined. Twenty-four hours after *oral* administration, 60-80% of the drug had been excreted in the feces, about 10% in the urine and 10-30% was found in the blood. Three days after *intravenous* administration, 78% of the drug was still present in the blood and tissues, and 22% had been excreted in the urine.

Absorption of pyrimethamine hydrochloride by the excised rat gut at physiologic pH amounted to less than 2%.

A variety of inorganic and organic salts of pyrimethamine were perfused in the rat intestine *in situ* for three hours. The amount of drug absorbed ranged from 10-20% with the inorganic salts (HCl, HBr, H_3PO_4) to 30-40% with the organic salts (acetate, succinate, glutamate). When the free base of pyrimethamine was perfused, 60% absorption was obtained. However, when the perfusate was passed through the stomach as well as the gut, absorption was reduced to 10-20%.

Enhanced antimalarial activity in infected mice and chickens was obtained with the oral administration of organic salts when compared with the free base and inorganic salts. The inconsistency observed in individual animals would suggest the use of parenteral administration or coated tablets to achieve desired and reproducible plasma levels.

Toxic manifestations of muscular fibrillation, convulsions and respiratory arrest are obtained with elevated plasma levels of pyrimethamine. In vitro, this compound alters epinephrine metabolism. At 10^{-5}M both the oxidation and methonylation of epinephrine appear to be inhibited. The toxicity of the drug may in part be due to increased levels of epinephrine.

DRUGS - TRIALS AGAINST INTESTINAL PARASITES

A total of over 300 cases of hookworm have been treated with bephenium (four different salts) employing a number of dosages and regimens. The more effective regimens, using the granular hydroxynaphthoate salt (Alcopara (R)) at 5 grams base per day, have produced worm reductions of 74 per cent (5 grams total base), 93 per cent (15 grams base), and 98 per cent (25 grams base). The drug is almost completely curative against Ascaris lumbricoides, and higher dosages appear also to have some effect against Trichuris trichiura. Trials of the newer drug dithiazanine iodide (Delvex(R)) against T. trichiura and other helminths continue to confirm the effectiveness of this drug; results against other helminths (hookworm and Ascaris) have not been encouraging. Use of this drug, alone and in combination with the anti-hookworm drug tetrachlorethylene, in the long term mass treatment of the population of an entire building has been carried out. The results are as yet incomplete. After the first 10 months of use of regularly repeated treatments with small doses, it appears likely that elimination of existing worm infections or elimination of transmission has not been achieved.

Puromycin, previously shown to be effective against asymptomatic infections of Endamoeba histolytica, cured four cases of amebic dysentery.

Epidemiological studies continuing on a defined population continue, after 5 years, to show a high proportion of persistence of Trichuris and hookworm. Entamoeba coli continues to be the only protozoan showing persistence for this extended period in a significant number of cases.

DRUGS AND NUTRITION AS RELATED TO SCHISTOSOMIASIS

Oral and parenteral regimens of 165 compounds were given to infected mice. Several compounds proved to be weakly schistosomacidal at toxic levels. Two of the compounds were more

promising and were studied intensively. The phenyl-piperazine (Hoechst S 688) previously found to be curative in mice and monkeys was found to have some prophylactic effect in dogs. An oral dose of the compound given on each of the three days following exposure prevented some of the S. japonicum from developing in dogs but this prophylactic effect was not observed in monkeys exposed to S. mansoni. The therapeutic index of S 688 was low in monkeys and dogs.

The second compound selected for trial was an antibiotic, S 1629. A regimen of several oral doses prevented one-half to three-fourths of the worms from developing in mice. In a prophylactic test, no eggs have been found in the feces of a treated monkey while they have been found in the non-medicated control.

Motile S. mansoni exposed in vitro to the active drugs Miracil D, stibophen, tartar emetic, and S 688 were depressed at a rate dependent on the drug and its concentration. Eserine produced spasmodic movements and paralysis comparable with the neuromuscular effect of this drug in other animals. The effects of these drugs were not reversed by fresh medium nor by atropine. Since many chemicals had the same killing effect on the parasites in vitro but were not active in vivo, the value of the in vitro test for screening of drugs for schistosomacidal activity is questioned.

The results to date indicate that stibophen (Fuadin) therapy of mature Schistosoma mansoni infections in mice is greatly enhanced by feeding an enriched semi-synthetic diet containing 30% protein in place of the usual commercial ration. The worm reduction on the enriched diet was 87%, 95%, and 65% in contrast to 5%, 12%, and 15% on the regular diet.

VIRUS-PARASITE ASSOCIATIONS A series of experiments with Nematospiroides dubius and LCM virus in mice to detect the presence of virus in intestinal contents, intestinal wall, feces, and in developed N. dubius larvae showed no significant viral content. This virus-parasite combination will probably not be productive of associations of the type sought in the current studies.

Simultaneous intraperitoneal inoculation of LCM virus and P. berghei parasites produced death in mice as early as if virus had been given intracerebrally; virus alone given intraperitoneally produced no deaths; nor could virus be recovered from the brain during 14 days of observation. This is indicative of a possible role of the parasite in delivery of the virus to the central nervous system. Further, whole blood derived from mice inoculated intraperitoneally with P. berghei and either intracerebrally or intraperitoneally with LCM virus can be shown to contain large amounts of virus. It was only occasionally possible to demonstrate virus in whole blood from

mice inoculated intracerebrally with LCM, and not at all when virus was given intraperitoneally. Thus the intercurrent P. berghei infection may be responsible for an otherwise absent or transient viremia. Other studies have shown no significant additive or sparing effect on the part of either the virus or the parasite when the two are given simultaneously in varying dilutions.

In preliminary studies of simultaneous SLE virus and P. gallinaceum in chicks, a combination of virus and parasite caused death 3 to 4 days earlier than with parasites alone. Virus alone appears not to cause significant disease in the chicks.

The virus of St. Louis encephalitis was taken up by the larvae of Aedes aegypti and a stepwise increase in virus titer was found in pupal mosquitoes up through eight days of incubation. The virus persisted into the imaginal stage of both male and female mosquitoes. The immature stages of Aedes aegypti developed higher virus titers and rates of infection than did Anopheles quadrimaculatus. The larvae of Anopheles quadrimaculatus and A. freeborni have not developed apparent infections after oral ingestion of the gametocytes of Plasmodium falciparum.

Serial No. NIAID-130
1. Parasite Chemotherapy
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Administration, research planning and coordination

Principal Investigator: G. Robert Coatney

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1 3/4
Professional:	3/4
Other:	1

Project Description:

This project furnishes certain technical, supervisory, and administrative services to all research projects in the Laboratory, as follows:

(a) Over-all planning of research and the coordination of research activities in the various Sections of the Laboratory.

(b) Integration of Laboratory research activities with clinical studies of the Clinical Center.

(c) Supervision over personnel, maintenance, travel, and correspondence.

(d) Requisitioning and supervision of equipment and supplies.

(e) Preparation of reports, budget estimates, and exhibits and other public relations materials.

(f) Maintenance of reference library, reprint files, card catalogues, and specimen collections.

(g) Consultatory services to individuals, commercial organizations, nongovernmental organizations, Government agencies including liaison activities with other branches of the Service, foreign governments, and international agencies.

(h) Reviewing, editing, and revising scientific and technical reports and manuscripts.

Research projects are reviewed quarterly. If necessary, they are discontinued or revised in accordance with current developments and needs. Some changes have been made in the research program of the Laboratory and some activities directed into new channels. Continued emphasis was placed on the fundamental aspects of problems in tropical parasitology and chemotherapy. These include the culture of insect tissues, intensive and extensive study of the development and chemotherapy of fixed tissue stages of certain mammalian malarias, the tissue culture of mammalian malarias, the lachesiology (fate) of drugs in the host, the development of drug extenders, the mechanism of drug action on parasites and the inhibiting effect of antimetabolites, and the carriage of viruses by parasites. Emphasis was also placed on the metabolic requirements of parasites and the chemistry of their disease-producing proclivities.

A cooperative study was inaugurated with Dr. W. Newton, LCAR, (Ser. No. NIAID-115), to test the germ-free host (mice) response to infection with P. berghei, and another with Dr. John Tobie, LI, (Ser. No. NIAID-148), on the application of fluorescent antibody techniques to the staining of human plasmodia for mass screening with a mechanical scanner.

Part B included - Yes.

Serial No. NIAID-130

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Coatney, G. R. and Greenberg, J. The effect of a diet deficient in Factor 3 on the course of Plasmodium berghei infection in mice. Proc. VI Internatl. Congress Trop. Med. & Mal. (in press).

Coatney, G. R. Introduction to Presidential Address by Lewis W. Hackett. Am. J. Trop. Med. & Hyg. (in press).

Honors and Awards relating to this project:

Visiting Professor, Department of Preventive Medicine and Public Health, School of Medicine, Howard University.

Visiting Lecturer on Tropical Public Health, Harvard School of Public Health.

Visiting Lecturer on chemotherapy, Malaria Eradication Training Center, Kingston, Jamaica.

Consultant to Chief, Section on Malaria Eradication, Pan American Sanitary Bureau.

Vice President, American Society of Tropical Medicine and Hygiene.

Member of Expert Advisory Panel on Malaria of the World Health Organization.

Serial No. NIAID-110-A
1. Parasite Chemotherapy
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies in human malaria

Principal Investigator: G. Robert Coatney

Other Investigators: Morton E. Getz, Martin D. Young, and Henry K. Beye

Cooperating Units: Pan American Sanitary Bureau and Bureau of Prisons

Man Years (calendar year 1959):

Total:	3 3/4
Professional:	1 1/4
Other:	2 1/2

Project Description:

Objectives:

To evaluate new antimalarial drugs and/or drug combinations and methods of administration applicable to mass chemotherapy as applied to malaria eradication.

Methods Employed:

Normal inmate volunteers at the Federal Penitentiary, Atlanta, Georgia are screened before they are admitted to the project. Volunteers are under the care of the resident investigator whether handled as outpatients or as inpatients. They take drugs as prescribed, submit to necessary laboratory procedures and to infection by bites of infected mosquitoes. Participation may be for 6 weeks to 2 months or up to one year depending on the nature of the project.

Major Findings:

Chesson strain of Plasmodium vivax.

Single doses as low as 75 mg. (base) of chloroquine (Aralen) per week will result in complete suppression of Chesson strain vivax malaria even under conditions of repeated infection

via mosquito bite. The present accepted dose is 300 mg. (base) per week.

Primaquine given as a single 30 mg. dose, weekly, or as a single 3.0 mg. dose, daily, beginning the day before infection by mosquito, did not prevent or suppress infection.

Primaquine is sporontocidal at a dosage as low as 1.5 mg., single dose, daily. Lots of mosquitoes fed on two occasions, 24 hours apart on 3 different patients, prior to the first dose of drug produced sporozoites in their salivary glands. Some lots fed daily after the first dose of primaquine produced guts positive for oocysts through day 10; however, sporozoites did not develop in the salivary glands in any lots fed after day 6.

Venezuelan strain of P. vivax.

This strain of malaria, obtained from the State of Merida in eastern Venezuela, was found to have a prepatent period of 13 to 17 days; the incubation period was also 13 to 17 days (data from 10 patients). This strain was found to be resistant to pyrimethamine.

Significance to Bio-medical Research and the Program of the Institute:

This country, along with other countries of the Western Hemisphere, is committed to a program of malaria eradication. In line with this commitment, the research program of this laboratory is directed in a large part toward fundamental problems in chemotherapy with special emphasis on finding the best way to use drugs in malaria eradication programs. The studies under way at the Atlanta installation are aimed at finding ways to apply the fundamental knowledge gained in the laboratory and answering problems posed by workers in the field.

Proposed Course of the Project:

This project will continue during the coming year with special emphasis on finding and assessing methods of mass chemotherapy and studying the biology of malaria found resistant to ordinary methods of eradication.

Part B included - No.

PHS-416
Individual Project Report
Calendar Year 1956

Part A.

Project Title: Comparison of quinacrine and other 3-aminoquinolines

Principal Investigator: William F. Campbell

Other Investigators: G. Robert Jeanes

Cooperating Units: None

Man years (calendar year 1956)

Total:	.11
Professional:	.11
Other:	.11

Project Description

Objectives:

To measure the effects of structural variations in 3-aminoquinolines with respect to antimalarial activity and toxicity and to find explanations for these effects.

Methods Employed:

Various 3-aminoquinolines are being compared with respect to antimalarial activity against Plasmodium gallinaceum and Plasmodium berghei and with respect to toxicity in mice, chickens, and monkeys.

Major Findings:

The toxicity and antimalarial activity of a supply of quinacrine tablets of Finland Stock has been studied. The drug, or tablets, is widely used in the campaign against malaria in Liberia. An aqueous extract of the contents of these tablets yielded a dark red solution which proved to have anti-malarial activity against P. gallinaceum equal to a sample of the new 3-quinuclidine synthesized in the United States and supplied by the Sterling-Winthrop Research Institute. However, the toxicity of quinacrine was 20% greater than the

sample obtained locally. The difference in toxicity appears to be due to a red substance which is easily separated from the active antimalarial in the Russian tablets.

Significance to Bio-Medical Research and the Program of the Institute:

The 8-aminoquinolines are the most useful curative drugs available for malaria caused by Plasmodium vivax which is of world-wide occurrence. The eradication of malaria may be hastened by a better understanding of the action of this group of substances.

Proposed Course of the Project:

The differences in activity will be studied to determine whether they are the result of variation in the fate of the compounds in the host or the result of differences in the susceptibility of the malarial parasite to the compounds.

If differences of the latter type are found, deductions about the structures of the enzymes and coenzymes involved in antimalarial activity will be made.

This line of study will be explored during a period of 3 or 4 months and for a longer period if it develops in an interesting way. The future course of the study depends partly on the availability of new 8-aminoquinolines.

Part 3 included - No.

Serial No. NIAID-131-A
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The mechanism of action of antimalarial drugs

Principal Investigator: Karl A. Schellenberg

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

To obtain a better understanding of the biochemical basis for the action of antimalarial drugs.

Methods Employed:

The parasite Plasmodium gallinaceum is being studied in vitro and in vivo, particularly regarding ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) synthesis, respiration, infectivity, and uptake of drug by the parasite. A strain of P. gallinaceum resistant to pyrimethamine is being developed.

Major Findings:

The results indicate that the drugs fall into groups on the basis of their mode of action. Thus, pyrimethamine and chloroguanide specifically inhibit DNA synthesis, the former under all conditions studied, and the latter only in vivo. The drug effect appears reversible only by thymidine, indicating that the drug inhibits the enzymatic conversion of deoxyuridine to thymidine.

Drugs with a quinoline or acridine nucleus (quinine, chloroquine, quinacrine) inhibit respiration, RNA, and DNA metabolism apparently nonspecifically through interference with energy conservation by the parasite. Studies with quinine in greater

detail reveal that the drug action is dependent on a high potassium ion concentration surrounding the parasite, such as occurs inside the red cell. Thus, the action of quinine and related drugs on the malarial parasite and heart muscle may be similar, intimately connected with potassium ion metabolism or transport.

Significance to Bio-Medical Research and the Program of the Institute:

Little is known of the primary mechanism of action of most drugs. Knowledge in this area could lead to a rational development of more effective chemotherapeutic agents, not only against malaria but against other diseases where drugs are useful, such as heart disease and arthritis in the case of the quinoline-acridine drugs, and neoplasms in the case of the pyrimethamine group.

Proposed Course of the Project:

Investigation along the lines outlined above will be continued and extended to other species, including human malaria. Further work will then be initiated in cell-free systems to determine more specifically the locus of action of the various drugs.

Part B Included - No.

Serial No. NIAID-131-B
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Mechanism of antimalarial action - effect on bacteria

Principal Investigator: Micah I. Krichevsky

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To determine the mechanism of inhibition of Pseudomonas sp. (Strain G4A) by pyrimethamine at the enzymatic level.

Methods Employed:

Growth rates of Pseudomonas sp. were determined by turbidimetric measurements. Utilization of substrates was determined by measuring gas evolution in the Warburg microrespirometer, by chromatographic and photometric methods.

Major Findings:

1. Pyrimethamine inhibits growth, glucose utilization, and pyruvate utilization to approximately the same extent. At the levels tested, formate utilization is not inhibited.

2. The system degrading pyruvate to an equimolar mixture of acetate and formate is the one inhibited.

3. Of many substances tested, pyruvate itself was the only material capable of reversing pyrimethamine inhibition.

Significance to Bio-Medical Research and the Program of the Institute:

An understanding of the biochemical nature of the action of drugs is a fundamental goal. Bacterial enzyme systems are accessible for study and serve as models for the analysis of drugs active against other organisms.

Proposed Course of the Project:

This project has been completed and the principal investigator has transferred to another Institute.

Part B included - No.

Serial No. NIAID-131-C1
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Experimental chemotherapy of helminthic diseases

Principal Investigator: George W. Lattermoser

Other Investigators: None

Cooperating Unit: Dr. B. Prescott, LID, NIAID-64

Man Years: (calendar year 1959):

Total:	2
Professional:	3/4
Other:	1 1/4

Project Description:

Objectives:

To discover new and more effective schistosomacidal compounds and the manner in which they selectively kill the worm parasites.

Methods Employed:

Mice are infected with Schistosoma mansoni and treatment begun, (a) immediately (prophylaxis) or (b) after 35 days (curative). The mice are examined 50 days or more after exposure for infection or for the presence of dead worms. In critical evaluation of drug activity, the liver and portal system are perfused and the live or dead worms are counted. Live mature worms removed from treated and untreated animals are observed in maintenance culture for activity and survival. Drugs are added to some of the cultures. Monkeys were experimentally infected with the same parasite; dogs with S. japonicum (Formosan). A test for either prophylactic or curative activity of promising compounds is conducted in the larger animals in a similar fashion to the mouse test except that fecal egg counts are also utilized as an indicator of the status of the infection.

Major Findings:

Oral and parenteral regimens of 16[illegible] chemical compounds were given to infected mice. Several of the chemicals proved to be weakly schistosomacidal at toxic levels but did not merit further study. However, two of the compounds were promising and were studied more intensively. The phenylpiperazine (Hoechst S 688) previously found to be curative in mice and monkeys was found to have some prophylactic effect in dogs. An oral dose of the compound given on each of the three days following exposure prevented some of the S. japonicum from developing in 4 dogs but this prophylactic effect was not observed in 6 monkeys exposed to S. mansoni. The therapeutic index of S 688 was low in monkeys and dogs.

The second compound selected for further trial was an antibiotic, S 1629. A regimen of several oral doses of this antibiotic prevented from one-half to three-fourths of the worms from developing in mice. In a prophylactic test in progress, no eggs have been found in the feces of a treated monkey while they have been found in the non-medicated control.

Motile S. mansoni exposed in vitro to the active drugs Miracil D, stibophen, tartar emetic, and S 688 were similarly depressed but at a rate dependent on the drug and its concentration. Eserine produced spasmodic movements and paralysis comparable with the neuromuscular effect of this drug in other animals. The effects of these drugs were not reversed by fresh medium nor by atropine. Since many chemicals had the same killing effect on the parasites in vitro but were not active in vivo, the value of the in vitro test for screening of drugs for schistosomacidal activity is questioned.

Significance to Bio-medical Research and the Program of the Institute:

Information available indicates that human schistosomiasis is increasing in many parts of the world especially where the practice of irrigation is growing. Current treatments for schistosomiasis are inadequate. A better drug is needed not only for treatment of individuals but also for mass usage in control programs.

Proposed Course of the Project:

Screening will continue in an effort to find more promising compounds. Moreover, an effort will be made to understand

how presently known compounds kill, *in vivo* and *in vitro*, and how their activity might be enhanced by diet or other means.

Part B included - Yes.

Serial No. NIAID-131-C

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Luttermoser, George W., Bruce, John I., and McMullen, Donald B. The prophylactic and curative activity of 1-Maleinyl-4-(3'-chloro-4'-methyl-phenyl)-piperazine (Hoechst S 688) in experimental schistosome infections. Am. J. Trop. Med. & Hyg. (Accepted for publication in next issue).

Honors and Awards relating to this project:

President of Helminthological Society of Washington, D. C. for 1960.

Serial No. NIAID-131-D
1. Parasite Chemotherapy
2. Chemotherapy
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The effect of nutrition on chemotherapy of parasitic diseases

Principal Investigator: George W. Luttermoser

Other Investigators: None

Cooperating Units: Dr. William DeWitt, LPD, NIAID-121-K

Man Years (calendar year 1959):

Total:	1
Professional:	1/4
Other:	3/4

Project Description:

Objectives:

To determine whether the nutrition of the host can influence the chemotherapy of parasitic infections.

Methods Employed:

Animals maintained on defined diets either enriched or deficient in certain components are exposed to a single species of parasite and the developing infection is treated in either the prepatent or patent period. The influence of the diet on treatment is determined by (1) the reduction of the number of parasites, (2) the condition of the parasites, (3) growth and survival of the host, and (4) the pathologic conditions developing in the host.

Major Findings:

The results of three experiments indicate the efficacy of stibophan (Fusdin) therapy of mature Schistosoma mansoni infections in mice is greatly enhanced by feeding the mice on enriched semi-synthetic diet containing 30% protein in place of the usual commercial ration. The worm reduction in mice on the enriched diet was 87%, 95% and 65% in contrast to 5%, 12%, and 15% on the regular diet.

Significance to Bio-medical Research and the Program of the Institute:

It may be possible to intensify the activity of drugs such as stibophen in man by modification of the diet. Furthermore, parasitic infections are prevalent in areas where malnutrition is common. Since there are many failures of treatment it is important to know whether malnutrition contributes to the poor results. It is also important that animals used in testing compounds for schistosomacidal activity be on rations providing optimum conditions for drug activity.

Proposed Course of the Project:

The present study is to be extended to determine the specific nutritional factors involved in the enhancement of the efficacy of stibophen in schistosomiasis. Other drugs used for the treatment of this and other parasitic diseases will be investigated.

Part B included - No.

Serial No. NIAID-131-E
Parasite Chemotherapy
Chemotherapy
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Physiological disposition and metabolism of the antimalarial drug, pyrimethamine

Principal Investigator: Leo E. Gaudette

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1 3/4
Professional:	1/2
Other:	1 1/4

Project Description:

Objectives:

To determine the physiological disposition and metabolism of this drug; to elucidate the physical and chemical properties influencing its absorption; and to re-evaluate its toxicology and pharmacology under the conditions listed in an effort to better define its use in a program for mass therapy.

Methods Employed:

Plasmodium berghei in the mouse and Plasmodium gallinaceum in the chick are the malaria strains used primarily. Pyrimethamine is employed as the free base and as inorganic and organic salts. In vivo and in vitro studies are accomplished in the mouse, rat, and rabbit. Specific chemical extraction methods are developed for the isolation of parent compound and/or metabolic products in urine, feces, blood, plasma, and tissues.

1. Physiological disposition of tritiated drug is determined in animals after oral and parenteral administration. Tissue distribution, plasma levels, excretion rates and metabolic products are determined by specific chemical methods and paper chromatography, assaying with proportional gas-flo counting or color reaction (if isotope dilution is employed).

2. The rate of absorption of the compound is determined in the rat by (a) perfusion studies in situ employing prolonged recirculation techniques and (b) 24-hour dialysis of excised rat gut segments.

3. Toxic manifestations observed are studied more critically at the enzyme level.

Major Findings:

The physiological disposition of radioactive pyrimethamine in the rabbit after oral and parenteral administration at a dose of 0.5 mg/kg was determined. Twenty-four hours after oral administration, 60-80% of the drug had been excreted in the feces, about 10% in the urine and 10-30% was found in the blood. Three days after intravenous administration, 78% of the drug was still present in the blood and tissues, and 22% had been excreted in the urine.

Absorption of tritiated pyrimethamine hydrochloride by the excised rat gut at physiologic pH amounted to less than 2%.

A spectrum of inorganic and organic salts of pyrimethamine were perfused in the rat intestine in situ for three hours. The amount of drug absorbed ranged from 10-20% with the inorganic salts (HCl, HBr, H_3PO_4) to 30-40% with the organic salts (acetate, succinate, glutamate). When the free base of pyrimethamine was perfused, 60% absorption was obtained. However, when the perfusate was passed through the stomach as well as the gut, absorption was reduced to 10-20%.

Enhanced antimalarial activity in infected mice and chickens was obtained with the oral administration of organic salts when compared with the free base and inorganic salts. The inconsistency periodically obtained with individual animals would suggest the use of parenteral administration or coated tablets to achieve desired and reproducible plasma levels.

Toxic manifestations of muscular fibrillation, convulsions and respiratory arrest are obtained with elevated plasma levels of pyrimethamine. In vitro, this compound alters epinephrine metabolism. At concentrations of 10^{-5}M both the oxidation and methylation of epinephrine appear to be inhibited. The toxicity of the drug may in part be due to increased levels of epinephrine.

Significance to Biomedical Research and the Program of the Institute:

The project is intimately associated with a larger project aimed at eradication of malaria. The purpose of the project is to

conduct basic studies on a widely used drug which sometimes fails to produce the desired result. Basic studies dealing with the lachesiology (fate) of the drug might well contribute to a better method of administration.

Proposed Course of the Project:

Studies dealing with the physiological disposition of pyrimethamine after oral and parenteral administration will be continued with particular emphasis on obtaining better absorption. In vitro studies will be included also.

Part B included - No.

Project No. MIAD-1311-F
Research Chemotherapy
Chemotherapy
Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemical pharmacology of the antimalarial drug chloroquine diphosphate

Principle Investigator: Leo E. Gaudette

Other Investigators: None

Cooperating Units: Edward D. Titus, G. R. Coatney

Man Years (calendar year 1959):

Total:	1 1/2
Professional:	1/2
Other:	1

Project Description:

Objectives:

To define metabolic pathways and enzyme systems active on antimalarial drugs; to determine mechanisms for prolonged antimalarial activity.

Methods Employed:

The antimalarial activity of drug combinations is tested in mice infected with Plasmodium berghei. In vivo and in vitro studies are accomplished in the mouse, rat, rabbit, and dog. Specific chemical methods are developed for the determination of parent compound and metabolic products in urine, feces, blood, plasma, tissues, and tissue preparations.

1. Malaria infection is maintained in mice by blood inoculations. The chemotherapeutic efficacy of drug combinations is determined by the course of the infection, as compared with controls.

2. Plasma levels in various species are determined on oxalated blood, by selective extraction and assay by spectrophotofluorometric techniques.

3. The metabolism of the antimalarial is determined in tissue preparations. Enzyme systems are characterized by classical and specialized biochemical techniques.

Major Findings:

An in vitro system for chloroquine metabolism was found in rabbit liver homogenates. Chloroquine was metabolized at a rate of about 45 per cent per hour per gram of tissue at pH 7.4 in an atmosphere of 95 per cent oxygen. In the presence of SKF 525-A, Lilly 18947, and pyrimethamine at concentrations of 1×10^{-4}M, chloroquine metabolism was inhibited 80, 90, and 100 per cent, respectively. Iproniazid and isoniazid had no effect on the in vitro system. The metabolic activity of liver homogenate was virtually lost after dialysis for 16 hours against distilled water at 5° C., but could be restored by the addition of a TPN-H generating system. One of the metabolic products is acetaldehyde and preliminary evidence suggests that both alkyl groups of the parent compound are oxidized in vitro.

Administration of these metabolic inhibitors to rabbits at pharmacological doses in combination with chloroquine gave rise to higher blood levels and a prolongation of the half-life of the antimalarial drug. However, in most cases, metabolic inhibition beyond 48 hours could not be achieved because of the rapid turnover of the prolonging agent.

Chloroquine was administered orally in combination with a metabolic inhibitor (SKF 525-A, Lilly 18947, pyrimethamine and isoniazid) to mice infected with P. berghei. It was found that after a single dose of chloroquine in combination with any one of the various inhibitors, the appearance of parasites in the blood was suppressed for a longer time than after treatment with chloroquine alone. The chloroquine-SKF 525-A combination at 5 mg/kg had four times the suppressive action of chloroquine alone. The mechanism of action in all cases proceeded by inhibition of chloroquine metabolism. None of the metabolic inhibitors employed had any antimalarial effect. On the contrary, some stimulated parasite development.

Significance to Bio-medical Research and the Program of the Institute:

The project is associated with a larger one aimed at the eradication of malaria. The purpose of the program is to find a drug or drug combination with prolonged action, maximum therapeutic value, and minimal toxicity.

Proposed Course of the Project:

1. Metabolic studies of antimalarial agents in general will continue *in vivo* and *in vitro*, particular emphasis being placed on suppressive agents. The enzyme systems and the metabolites of antimalarials will be investigated further, giving consideration to the possible inhibition of the enzyme systems, potentiation of antimalarial activity of the parent compound, and possibly metabolic product.

2. Other metabolic inhibitors of chloroquine metabolism but with longer action sought.

Part B included - No.

Serial No. NIAID-132-A
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Studies on human malaria

Principal Investigator: Martin D. Young

Other Investigators: John Paul Duffy and Aimee Wilcox

Cooperating Units: South Carolina State Hospital

Man Years (Calendar year 1959):

Total:	4 1/3
Professional:	1 1/3
Other:	3

Objectives:

To add to the biological knowledge of malaria by observations on induced malaria and experimental studies. To determine the sporontocidal activity of antimalarial drugs and how developed resistance influences transmission by mosquito vectors. To study the development and inheritance of resistance to drugs by the parasites. To determine if the development of resistance to insecticides by mosquitoes alters their ability to transmit malaria. To serve as probably the only source in the nation for established strains of malaria for use on a nationwide basis for therapy of neurosyphilis, nephrosis, Parkinsonism, and for experimental malaria studies.

Methods Employed:

Malaria is induced in neurosyphilitic patients and in volunteers under normal and experimental conditions. The patent infections are challenged by drugs. When resistance occurs measurements are made of its characteristics. Mosquitoes are allowed to bite patients with induced infections before and at intervals after drug administration. The resulting infections in the mosquitoes

are correlated with the presence or absence of drug resistance of the malaria infection in the human host. Insecticide-resistant and normal mosquitoes are fed simultaneously for comparison of ability to transmit malaria.

Major Findings:

Primaquine given 3 mgm daily had a continuous sporontocidal effect, beginning several days after the first dose, upon Plasmodium vivax and P. falciparum infections. Current work with 1-1/2 mgm daily indicates the same effect upon P. falciparum.

A strain of P. vivax malaria, from an area in Venezuela which had been under mass suppression with pyrimethamine, was induced in two patients. The ineffective response to challenges of 50 and later 100 mgm of the pyrimethamine suggests that drug resistance had developed under field conditions.

The enzyme succinic dehydrogenase was found in the gut of Anopheles quadrimaculatus.

The relative ability of normal and insecticide-resistant strains of A. quadrimaculatus and A. albimanus are being investigated but the data so far are not sufficient for conclusions.

A compilation of reported P. ovale infections indicates a great increase of recorded cases. Hundreds of cases have now been reported with prevalence rates up to 5 per cent in some populations. This is in contrast with the 105 cases which were reported as the total number of known cases in 1949. The significance of this apparent increase is being studied.

Significance to Bio-Medical Research and the Program of the Institute:

As the number of important vectors developing resistance to insecticides is increasing, it is important to know if this alteration affects the ability of the mosquito to transmit malaria or other diseases.

The development of pyrimethamine resistance by human malaria parasites indicates the need of basic research on the biology of the human malaria parasite. The demonstration of sporontocidal action of small frequent doses of primaquine suggests experimental chemotherapeutic trials to determine the practicality of such use in malaria eradication programs.

Proposed Course of the Project:

To more fully explore the vectorial abilities of insecticide-resistant mosquitoes with both normal and drug resistant malarias: Cooperative projects will investigate the prophylactic and suppressive action of regular doses of antimalarial drugs, such as primaquine, with the aim of practical use in malaria eradication programs. Basic biological studies on host-parasite relationships of malaria in man and in mosquito will be pursued, such as the longevity of P. malariae infections and the characteristics of foreign strains of malaria. Important strains of malaria will be added to those now in the local repository, which apparently is the only existing one for the preservation of malaria.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Young, M. D. and Burgess, R. W. Pyrimethamine resistance in Plasmodium vivax malaria. Bull. Wld. Hlth. Org. 20:27-36. 1959.

Burgess, R. W. and Young, M.D. The development of pyrimethamine resistance by Plasmodium falciparum. Bull. Wld. Hlth. Org. 20:37-46. 1959.

Jeffery, G. M., Young, M. D., Burgess, R. W., and Eyles, D. E. Early activity in sporozoite-induced Plasmodium falciparum infections. Annals of Trop. Med. Parasit. 53:51-58. 1959.

Young, M. D. Book Review: Davey's - A guide to human parasitology. Am. J. Trop. Med. Hyg. 8:200. 1959.

Young, M. D. Chemotherapeutic agents and malaria eradication. Proceedings of Sixth International Congresses on Trop. Med. and Mal. (In press.) 1959.

Garnham, P. C. C., Jeffery, G. M., and Young, M. D. Preservation of strains of malaria parasites. Proceedings of Sixth International Congresses on Trop. Med. and Mal. (In press.) 1959.

Young, M. D. The effect of small doses of primaquine upon malaria infections. (In press. Indian J. Mal.) 1959.

Young, M. D. MALARIA - A story of scientific exploration and achievement. (In press. Bull. S. C. Acad. Sci.) 1959.

Honors and Awards relating to this project:

For Dr. Martin D. Young:

Editor, Pro Tem., The American Journal of Tropical Medicine and Hygiene.

Member, Expert Advisory Panel on Malaria, World Health Organization.

Serial No. NIAID-132-B
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Chemotherapy and epidemiology of intestinal parasites in a mental institution

Principal Investigator: Martin D. Young

Other Investigators: Geoffrey M. Jeffery

Cooperating Units: South Carolina State Hospital

Man Years (calendar year 1959):

Total:	3
Professional:	1
Other:	2

Project Description:

Objectives:

To find adequate drugs for the prophylaxis and treatment of parasitic infections which constitute serious health problems in certain types of mental patients as well as in more normal populations; to determine the relationship of parasite load to psychological or psychiatric considerations in the patients; also to investigate factors influencing parasite spread and persistence among patients.

Methods Employed:

Various segments of the institutional population are examined to determine qualitatively, and quantitatively when possible, their parasitic infections. Promising compounds in varying dosages and regimens are tried against selected cases; post-treatment evaluation determines the effectivity of the drug against the various parasites. Occasionally careful pre- and post-treatment psychological, psychiatric and physical evaluations are done to determine the possible effect of parasite removal. The results are based upon 2000 patient-drug (individual regimens) trials.

Major Findings:

Extensive trials of four salts of a newer drug, bephenium, have been done, continuing the initial major controlled investigation of this drug. A total of over 300 cases of hookworm have been treated with a number of dosages and regimens. The more effective regimens, using the granular hydroxynaphthoate salt (Alcopara(R)) at 5 grams base per day, have produced worm reductions of 74 per cent (5 grams total base), 93 per cent (15 grams base), and 98 per cent (25 grams base). The drug is almost completely curative against Ascaris lumbricoides, and higher dosages appear also to have some effect against Trichuris trichiura. Trials of the newer drug dithiazanine iodide (Delvex(R)) against T. trichiura and other helminths continue to confirm the effectiveness of this drug against the former; results against other helminths (hookworm and Ascaris) have not been encouraging. Use of this drug, alone and in combination with the anti-hookworm drug tetrachlorethylene, in the long term mass treatment of the population of an entire building has been carried out. The results are as yet incomplete. After the first 10 months of use of regularly repeated treatments with small doses, it appears likely that elimination of existing worm infections or elimination of transmission has not been very successfully achieved.

Puromycin, previously shown to be effective against asymptomatic infections of Endamoeba histolytica, cured four cases of amebic dysentery.

Epidemiological studies continuing on a defined population continue, after 5 years, to show a high proportion of persistence of Trichuris and hookworm. Entamoeba coli continues to be the only protozoan showing persistence for this extended period in a significant number of cases.

Significance to Bio-Medical Research and the Program of the Institute:

Certain types of institutions, such as mental hospitals, are reservoirs of parasitic infections and some of the occupants are adversely affected by heavy worm infections. The finding of prophylactic and curative drugs for these infections is important not only for institutions but for normal populations where parasitism is a health problem. The findings are of importance in other parasitic disease problems, especially in developmental chemotherapy and in control and eradication programs. The possibility of improving the adjustment of the mental health by removal of worm infections is of importance.

Proposed Course of the Project:

The screening of promising compounds in the search for better parasitocidal drugs will be continued. Various dosages and regimens of the newly found effective drugs against hookworm and Trichuris will be tried in an effort to produce better means for treatment and prophylaxis of these infections in mental institutions and in more normal groups. Investigations are planned on the mode of action of drugs on parasites. Efforts will be made to explain the failure of very effective drugs to produce radical cures, the failure of repeated treatments to effect the same high worm reductions as initial treatments, and other similar problems. Further investigations on the relationship of parasite load to psychological, psychiatric, and physical considerations in the patients will be continued on a limited scale.

Part B included: Yes

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Young, M. D., Jeffery, G. M., Freed, J. E., and Morehouse, W. G., Bephenium, a new drug active against human hookworm. J. Parasit. 44:611-612. 1959.

Wolcott, G. B. The chromosomes of Diphyllobothrium ursi. J. Parasit. 45:378 and 384. 1959.

Young, M. D. The treatment of symptomatic amebiasis with puromycin. Antibiotic Med. and Clin. Therapy. VI:222-223. 1959.

Jeffery, G. M. A three-year epidemiological study of intestinal parasites in a selected group of mental patients. (In press. Am. J. Hyg.)

Honors and Awards relating to this project: None

Serial No. NIAID-152-C
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: The mechanism of drug action upon human parasites

Principal Investigator: Kenneth O. Phifer

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Objectives:

To determine the place of action of drugs upon malaria and intestinal parasites of man. The study of the action of drugs on malaria includes the mechanism and locale, the development of resistance to drugs and a search for methods to avoid the development of resistance or how to overcome it. Similar studies are planned on the developmental chemotherapy of other parasites, especially the worms of the intestinal tract.

The initial work is on the nucleic acids of Plasmodium in an effort to determine whether the nucleic acid composition and level is any different in parasites not exposed to chemotherapeutic agents, parasites exposed to drugs and found to be drug-sensitive and parasites found to be drug-resistant. To determine whether resistant parasites possess mechanisms of nucleic acid synthesis, which differ from those of normal parasites. To determine whether nucleic acid levels might be diagnostic indices of infection in the mosquito before morphological evidence is available.

Methods Employed:

The nucleic acids of Plasmodium gallinaceum, Plasmodium berghei and the malaria parasites of man are being studied with respect to the various nucleotide constituents and to the relative quantities of ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) in the parasitized host cells as compared with nucleic acid content of non-parasitized host cells. Mosquitoes and red blood cells are homogenized and fractionated into perchloric acid soluble compounds, phospholipids, RNA and DNA. The RNA is quantitatively measured by the orcinol reaction and the DNA is analyzed by the diphenylamine procedure.

Qualitative analysis of the nucleic acids is carried out through the use of paper chromatography and paper electrophoresis. Identification of constituents is effected through comparison with chromatograms and electrophoretic strips of standardized nucleotide mixtures.

Major Findings:

Most of the time has been spent in developing and standardizing procedures of biochemical analysis. Standard chromatographic patterns were obtained for the major nucleic acid constituents; these can be used later as reference points for comparison with the components of parasite nucleic acids.

Preliminary experiments have shown that in the case of Plasmodium gallinaceum, the DNA content of infected cells appears to be no different than that of uninfected cells. On the other hand, the RNA content of infected red cells is five times that of uninfected chicken erythrocytes.

Significance to Bio-Medical Research and the Program of the Institute:

Since several antimalarial agents have chemical structures similar to those of components of nucleic acids, it seems advisable to determine whether these drugs affect the parasite's basic nuclear compounds. It is felt that if it were demonstrated that nucleic acids are altered by antimalarials, this clarification of mechanism of drug action would provide a more logical rationale for the synthesis of new drugs.

Resistance of parasites to drugs is a serious world problem with respect to malaria eradication. Since resistance has been shown to be under genetic control in many instances, demonstration

of changes in nucleic acid composition in response to exposure to a drug should be of value in determining mechanisms of resistance. Once mechanisms of resistance are delineated, the problems associated with combatting drug resistance might be more readily solved.

A corollary aim of this project is the attempt to develop a biochemical means of detection of infection of mosquitoes before morphological stages in the parasite's life cycle are evident. If changes in nucleic acid levels or composition could serve as reliable indices of infection, some insight would be gained as to the changes occurring and the experimental procedures would be shortened.

Proposed Course of the Project:

Studies of normal malaria parasites will be continued in an attempt to obtain standards of reference. This will involve both qualitative and quantitative analyses of nucleic acids.

In addition, parasites from donors treated with various drugs will be compared with untreated parasites with respect to nucleic acid composition and relative level.

Parasite strains resistant to drugs will be compared with those shown to be sensitive to the drugs.

Studies of RNA and DNA fractions of parasites will be studied in relation to their possible antigenic properties both before and after therapy.

Other branches of metabolism will be studied in the attempt to develop an efficient biochemical index of malaria infection in the mosquito. These extended metabolic studies should also add to the information concerning mechanisms of drug action.

Part B included: No.

Serial No. NIAID-132-D
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Virus-parasite association

Principal Investigator: Geoffrey M. Jeffery

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1 1/2
Professional:	1/2
Other:	1

Project Description:

Objectives:

To determine experimentally if parasites might be associated with some of the virus diseases of man or animals as vectors or in some other capacity. If experimental evidence establishes such relationships between parasites and viruses, further objectives are to study such relationships under natural conditions and to investigate known virus infections and known populations to determine existing relationships. This includes the study of possible intestinal viruses among institutionalized mental patients. To investigate the possibility that certain parasites may themselves be afflicted with harmful virus infections. To investigate the effects of parasitic infections upon virus infections and vice versa.

Methods Employed:

Small animals and their common parasites are used in attempts at transmission of viruses. Viruses, such as LCM, polio, St. Louis encephalitis, rabbit papilloma and fibroma are maintained in small animals by serial transmission, providing infective virus material for experimental work. Besides the common helminth parasites of

mice, Nippostrongylus muris, Nematospiroides dubius, Hymenolepis nana, and others, the malaria parasites Plasmodium berghei and P. gallinaceum are also maintained as experimental parasites. Viruses and parasites are brought together in various ways, such as by simultaneous infection in animals, during cultivation of the free-living stages, or by simultaneous exposure of the host to infective parasite stages and virus suspensions. Tissue culture or small animals are used for detection of virus infection carried by parasites from virus-infected animals, for virus detection in parasites suspected of harboring viruses in nature, and for detection of virus infection in arthropods. Incidence of intestinal viruses and their possible association with parasites in institutionalized mental patients is studied with animal inoculation, tissue culture, and other means.

Major Findings:

Completed was a series of experiments designed to determine, during simultaneous infection with Nematospiroides dubius and LCM virus in mice as compared with virus alone, the possible presence of virus in intestinal contents, intestinal wall, feces and in developed N. dubius larvae. None showed significant viral content, indicative that this virus-parasite combination will probably not be productive of associations of the type sought in the current studies.

Studies with association of LCM and SLE viruses with Plasmodium berghei in mice, and SLE virus with P. gallinaceum in chicks, have yielded results of interest. Simultaneous intraperitoneal inoculation of LCM virus and P. berghei parasites produced death in mice as early as if virus had been given intracerebrally; virus alone given intraperitoneally produced no deaths; nor could virus be recovered from the brain during 14 days of observation. This is indicative of a possible role of the parasite in delivery of the virus to the central nervous system. Further, whole blood derived from mice inoculated intraperitoneally with P. berghei and either intracerebrally or intraperitoneally with LCM virus can be shown to contain large amounts of virus. It was only occasionally possible to demonstrate virus in whole blood from mice inoculated intracerebrally with LCM, and not at all when virus was given intraperitoneally. Thus the intercurrent P. berghei infection may be responsible for an otherwise absent or transient viremia. Other studies have shown no significant additive or sparing effect on the part of either the virus or the parasite when the two are given simultaneously in varying dilutions.

Preliminary studies on the combination of SLE virus with P. berghei have failed to show either the transport of virus or the enhancement of viremia seen in the LCM-berghei combination.

Preliminary studies of simultaneous SLE virus and P. gallinaceum in chicks show indications either of enhancement of parasitic effect, or of activation of the virus as a fatal infection. Virus alone appears not to cause significant disease in the chicks. A combination of virus and parasite caused death 3 to 4 days earlier than with parasites alone.

Significance to Bio-Medical Research and the Program of the Institute:

Many problems associated with the transmission of viruses in human and animal populations remain unsolved. New viruses in mammals and arthropods are continually being discovered. There are suggestions in the literature and theoretical possibilities that parasites may act as reservoirs and agents of transmission for viruses of importance to man. Elucidation of association of viruses and parasites, both experimental and natural, will do much toward bringing about a more complete understanding of the epidemiology of diseases produced by such organisms, alone or in combination.

Proposed Course of Project:

The general course of the project will be changed little. Repetition and expansion of the experiments combining nematode parasites and viral agents in the vertebrate host, during free-living stages, and in culture, will be carried out. An increased amount of effort will be directed toward the association of viruses and malaria parasites, and possibly other protozoans. Further refinement of viral detection methods will be investigated and incorporated in the work when practicable. Most of the experimental work planned is in the realm of artificial viral-parasite associations, but the possibility of extension of the studies to naturally occurring associations will be always in mind and such studies will be pursued as opportunities arise.

Part B included: Yes

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project: None

Honors and Awards relating to this project:

Dr. Geoffrey M. Jeffery:

Editor of the Bulletin of the Association of Southeastern Biologists.

1959 Recipient of the Bailey K. Ashford Award.

Serial No. NIAID-132-E
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development of human pathogens in immature insects.

Principal Investigator: William E. Collins

Other Investigators:

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

To determine experimentally to what extent immature stages of arthropods will support growth of pathogenic organisms with particular emphasis on the viruses and protozoans in mosquito larvae. If experimental evidence indicates the growth or maintenance of these microorganisms in insect larvae, investigations will be made to determine the possible role larval infection plays in the transmission of these infections to man.

Methods Employed:

The larvae of mosquitoes belonging to several different genera are exposed to virus suspensions under varying conditions. The quantity of virus present in larvae, pupae, and adults subsequent to the exposure is determined by small animal inoculations. Transmission of the virus by the adult mosquitoes is made using small animals and blood pools. Larvae of mosquitoes are likewise exposed to Plasmodium sp. and examinations made subsequently for the presence of the parasites.

Major Findings:

The virus of St. Louis encephalitis was taken up by the larvae of Aedes aegypti and a stepwise increase in virus titer was found in pupal mosquitoes up through eight days of incubation. The virus persisted into the imaginal stage of both male and female mosquitoes. The immature stages of Aedes aegypti mosquitoes develop higher virus titers and rates of infection than did Anopheles quadrimaculatus mosquitoes. The larvae of Anopheles quadrimaculatus and A. freeborni have not developed apparent infections after oral ingestion of the gametocytes of Plasmodium falciparum.

Significance to Bio-Medical Research and the Program of the Institute:

The presence of viruses and protozoans in mosquito breeding pools as a result of improper sanitation procedures or by the death and decomposition of infected animals and arthropods poses the possibility that these organisms may be taken up by the mosquito larvae and subsequently transmitted by the adults. Information on such larval infection will add to a more complete understanding of the epidemiology of a number of arthropod-borne diseases.

Proposed Course of the Project:

The relative ability of mosquito larvae of different genera to take up the virus of St. Louis encephalitis and the effect of initial virus titer, incubation temperature and larval instar on the rate of infection, virus titer and adult transmission will be tested. Larvae of Anopheles, Aedes, and Culex mosquitoes will be exposed to sporozoites of Plasmodium gallinaceum and P. vivax to determine the possible uptake of this form of the parasite and subsequent transmission by adults.

Part B. included: No

Serial No. NIAID-132-F
1. Parasite Chemotherapy
2. Epidemiology
3. Columbia, S. C.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Insect tissue culture

Principal Investigator: William E. Collins

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To establish insect tissues in culture in order to better study those factors necessary for insect growth and metamorphosis and the effect of microorganisms on insect tissues. To determine which of the viruses, protozoans or helminths will persist, develop, or multiply in the different insect tissues grown *in vitro* using both natural vectors and unrelated species.

Methods Employed:

Tissues and cells from moth larvae and pupae and from mosquitoes are removed and set up in experimental culture media using several different culture methods. The presence of mitoses, tissue contractility or general cell condition are used as indications of tissue growth or maintenance. Viruses are added to such cultures and periodic samples collected and titrated in small animals. Protozoans are added and microscopic examinations made to determine persistence or development of the parasites. Guts of mosquitoes previously infected with *Plasmodium* sp. are removed and set up in culture. Periodic examination and measurement of the parasite development are made.

Major Findings:

To date, only blood cells and cells of the ovariol sheath of caterpillars have undergone mitotic division in culture. The virus of St. Louis encephalitis was maintained in cultures of third instar larval blood cells from the catalpa sphinx (Ceratomia catalpae) for 120 hours. A number of mosquito tissues have been maintained in healthy condition for several weeks, but no mitosis observed.

Significance to Bio-Medical Research and the Program of the Institute:

Insects serve as alternate hosts for a number of virus and protozoan diseases of man. Insect tissue culture would offer the opportunity to study these parasites and their growth requirements, in vitro, and thus quite possibly shed new light on parasite growth requirements, arthropod tissue specificity and reasons why many human pathogenic viruses will multiply in the insect with no visible or apparent pathology. These studies may also yield information on those factors controlling the ability of insect vectors to transmit virus and protozoan diseases whereas closely related species of insects do not.

Proposed Course of the Project:

Blood cells, brain cells, fat body and other tissues from mosquito larvae, pupae, and adults will be established in culture and tested for their ability to support the virus of St. Louis encephalitis. Guts from mosquitoes infected with Plasmodium vivax and P. gallinaceum will be set up in different culture media in an attempt to determine those factors necessary for the development of sporozoites in vitro. Further studies on the effect of virus on caterpillar blood cells and ovariol tissue will be made.

Part B included: No.

Serial No. NIAID-133-A
1. Parasite Chemotherapy
2. Section on Cytology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the biology of the exoerythrocytic phases of primate malaria.

Principal Investigator: Don E. Eyles

Other Investigators: Robert L. Ingram, Nell C. Owen, Frances E. Jones and Clinton S. Smith

Cooperating Units: None

Man Years (calendar year 1959):

Total:	2-3/4
Professional:	1-1/2
Other:	1-1/4

Project Description:

Objectives:

To determine if the reported occurrence of exoerythrocytic parasites of primate malaria in the liver is correct. To extend the knowledge of the exoerythrocytic stages, determine the type of cells parasitized, and trace the development from sporozoite to mature schizont. To determine if the liver is the sole tissue parasitized. To determine the origin and nature of the late tissue stages and to determine their relationship to relapse. To define in detail quantitative relationships in the exoerythrocytic cycle. To study the exoerythrocytic cycle as it is related to the immune processes of the monkey host. To determine factors influencing the degree of exoerythrocytic infection. To provide materials and correlative information to accompany projects on growth of malaria parasites *in vitro* and the chemotherapy of malaria (133-B and 133-C).

Methods Employed:

Plasmodium cynomolgi, which is closely related to *P. vivax*

of man, is maintained in Macaca mulatta monkeys. Strains of P. coatneyi (also a simian vivax-type parasite) and P. inui related to human P. malariae) are also maintained. Anopheles mosquitoes of several species are produced and mosquitoes infected in order to produce very large numbers of infected mosquitoes. Salivary glands are dissected from these mosquitoes and injected intravenously in order to produce patent liver infection.

At predetermined intervals, liver specimens are taken by laparotomy. Histological studies are made on living and stained material. Flourescent-antibody as an aid in locating parasites is employed, and other labelling methods shall be used as necessary.

Major Findings:

A series of three experiments into monkeys has been done in which uninfected glands in numbers up to 2,026 pairs have been injected into monkeys without producing artifacts which might be confused with exoerythrocytic parasites. The same animals showed abundant parasites when smaller numbers of infected glands were injected. Successful experiments involving 35 monkeys have given consistent results; that is, injection of infected glands is followed by a liver exoerythrocytic infection commensurate with the number of glands injected. The number of parasites examined has been several thousand.

Morphological studies of two strains of P. cynomolgi have been made which in addition to their own interest will be useful as guidelines in chemotherapy studies and in studies on in vitro growth. Growth has been seen from the fourth through the ninth day and mature parasites have been observed as late as the 120th day. Reaction of the host has been found to be lacking in most monkeys, but reaction to the parasites has been studied in one animal. Size changes through the cycle have been studied, and it was found that on a given day the size was consistent. Distribution of parasites was found to be uniform throughout the liver and fit the Poisson distribution well.

Previous observations on the relationship of the number of exoerythrocytic forms and the subsequent parasitemia have been confirmed. The effect of cortisone on the liver infection has been studied. Exoerythrocytic parasites have been seen after monkeys were bitten by infected mosquitoes, but were not injected with glands.

Significance to Bio-medical Research and the Program of the Institute:

Knowledge of the exoerythrocytic stages of malaria is essential in a program of chemotherapeutic study as it is these stages which must be eliminated if antimalarials are to be used in malaria eradication. Although work accomplished to date indicates beyond any reasonable doubt that the findings are valid, there remain unelucidated portions of the exoerythrocytic cycle. Study of the simian species is especially appropriate as the parasites are very similar to those of man.

This project is coordinated closely with others on malaria at this laboratory including projects on in vitro cultivation and the effect of drugs directly on the parasite. (NIAID-133-B and C). It is another phase of the integrated malaria study of the Laboratory of Parasite Chemotherapy.

Proposed Course of Project:

During the coming year especial emphasis shall be placed on the relationship of immunity to the exoerythrocytic cycle. The project shall continue to be a source of material for in vitro work. The difficult problem of the development during the first three days after sporozoite injection shall be studied using labelling methods. The course of events leading up to relapse shall be studied.

Part B included - Yes.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

The following publications are included under this project even though they resulted from a previous project which was replaced by the present one. The reason for this is that the NIH list of publications is made up from Part I and these papers would not be included otherwise.

Jones, Frances E., Melton, Marjorie L., Lunde, Milford N., Eyles, Don E. and Jacobs, Leon. Experimental toxoplasmosis in chickens. J. Parasitol., 45(1):31-37, 1959.

Eyles, Don E., Gibson, Colvin L., Coleman, Nell, Smith, Clinton S., Jumper, John R. and Jones, Frances E. The prevalence of toxoplasmosis in wild and domesticated animals of the Memphis region. Am. J. Trop. Med. & Hyg., 8(4):505-510, 1959.

Eyles, Don E. and Coleman, Nell. The effect of metabolites on the anti-toxoplasmic action of pyrimethamine and sulfadiazine. Accepted for publication in the Am. J. Trop. Med. & Hyg.

Gibson, Colvin L. and Jumper, John R. The prevalence of canine toxoplasmosis in Memphis, Tennessee. Accepted for publication in the J. Parasitol.

Honors and Awards relating to this project None.

Serial No. NIAID-133-B
1. Parasite Chemotherapy
2. Section on Cytology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the *in vitro* growth of malaria parasites, especially exoerythrocytic stages.

Principal Investigator: Robert L. Ingram

Other Intestigators: Don E. Eyles, Frances E. Jones, Nell C. Owen and Clinton S. Smith

Cooperating Units: None

Man Years (calendar year 1959):

Total:	3-3/4
Professional:	2
Other:	1-3/4

Project Description:

Objectives:

To devise methods of maintaining malaria parasites *in vitro*, either in growing cell cultures or in organized tissue maintained *in vitro*. To study at the molecular level certain aspects of the biochemistry of the malaria parasite, the pharmacological actionof chemotherapeutic agents on the parasite, and the effect of the parasite on the host cell metabolism.

Methods Employed:

Standard cell and organ culture methods are employed, being modified when necessary. Material for culture is obtained from the closely coordinated project on the study of exoerythrocytic parasites. Appropriate biochemical methods are used in metabolic studies of both parasitized and unparasitized cells.

Major Findings:

Cell lines have been established from monkey liver and other sources. Considerable success has been achieved in maintaining organized liver tissue *in vitro*. Good survival of

organized fragments of adult monkey tissue has been obtained for as long as 10 days which is sufficient time for P. cynomolgi and other malaria parasites to complete an exoerythrocytic cycle. The ability to maintain liver is a major step and is basic to studies of the parasites in vitro.

Exoerythrocytic stages of P. cynomolgi have been grown in vitro for two and four days from fragments of tissue taken from heavily infected monkeys. The parasite increased greatly in size during the period of in vitro maintenance. The cytology of the parasites compares well with normal in vivo parasites. This may represent the first in vitro cultivation of mammalian exoerythrocytic parasites.

A monkey was injected with cultured material taken from an infected monkey at seven days after sporozoite inoculation, then maintained in culture for two additional days. The monkey developed an infection.

A large number of experiments have been done in which cultures of cell lines or organized tissues have been inoculated with malaria sporozoites. The experimental conditions have been altered in various ways. In some instances, sporozoites were introduced into cultures by the bite of mosquitoes through membranes through a specially devised technique. So far, no patent infections have been produced from sporozoites.

Preparatory to the preceding, studies were done on the elimination of the contaminating microbial organisms found in the mosquitoes with some success. Achromycin and mycostatin were found useful having an effect on the contaminants while sparing the cells and the sporozoites. Penicillin and streptomycin were not effective against the mosquito microflora.

As a tool for use in in vitro as well as in vivo studies, antibodies to a sporozoite antigen of P. gallinaceum have been produced in rabbits. After conjugation with fluorescein isothiocynate blood forms were stained with apparent success. Further tests are needed but these preliminary results indicate that this technique may be used in malaria studies to detect organisms. The problem is of some interest also as the antigenicity of sporozoites has been a point of question.

Significance to Bio-medical Research and the Program of the Institute:

The use of in vitro methods to study the malaria parasite, particularly the exoerythrocytic stages, will make possible studies on a cellular or possibly a molecular level on physiology of the parasite. These studies could pave the way for investigators with other organisms where it was desirable to study the intimate host-parasite relationship with organized tissue without concomitant infections with other agents and without the effects of humoral

factors, etc. Basic information may be obtained on the action of antimalarial drugs. Information developed may be applicable to other parasitic infections of man and animals. Metabolic studies of cells in tissue culture either parasitized or nonparasitized, may provide new information on cellular functions.

Proposed Course of Project:

The project is now in its earliest stages. The efforts during the coming year will be directed toward the establishment of cell lines and the growth of parasites in these cell lines, and toward improving methods of maintaining infected liver tissue in vitro. Studies of cellular function in cell culture and organ culture will be made. Once practical methods of maintaining the parasite in vitro are developed, the effort will be concentrated on biochemical studies of the physiology of the parasite and the pharmacological action of drugs upon the parasite.

Some attempts may be made to culture insect tissues in order to study aspects of the parasite in the insect host. All phases of the in vitro cultivation project will continue to be closely correlated with other aspects of the malaria study at this laboratory.

Part B. included - No.

Serial No. NIAID-133-C
1. Parasite Chemotherapy
2. Section on Cytology
3. Memphis, Tennessee

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Chemotherapeutic studies of the direct effect of drugs and antimetabolites on the exoerythrocytic stages of primate malarias.

Principal Investigator: Don E. Eyles

Other Investigators: Robert L. Ingram, Nell C. Owen, Clinton S. Smith

Cooperating Units: None

Man Years (calendar year 1959):

Total	:	2-1/2
Professional	:	1-1/2
Other	:	1

Project Description:

Objectives:

To determine the direct effects upon exoerythrocytic parasites of primate malarias of established antimalarial drugs. To describe morphological and histochemical changes and relate these to mode of action. To determine dosages necessary for effect. To study effect as related to time of administration; that is, before infection and at various times during infection. To study possible synergists. To study the reaction of the primate host to killed or damaged parasites. In the case of incomplete effects, to determine how parasites survive treatment and produce relapse.

To study the direct effect of various substances of known metabolic or antimetabolic activity on primate exoerythrocytic stages. To relate findings to physiological processes of the parasites, with the ultimate objective of obtaining leads toward development of more effective drugs. It is quite possible that incomplete effects, not detectable by indirect studies of relapse, might be seen directly, and provide information useful in future work.

To study the direct effect on exoerythrocytic stages of repository-type drugs, particularly repository forms of 8-amino quinolines. To study the effect of drugs on resistant strains of primate malarias, to determine if resistance of erythrocytic stages is associated with resistance of the exoerythrocytic stages or vice versa.

Methods Employed:

Plasmodium cynomolgi, the vivax-type parasite of macaques, is the principal parasite used; however, we plan to use the malariae-type, Plasmodium inui, as suitable techniques are developed. For chemotherapeutic studies, infections are established in the livers of monkeys by the intravenous inoculation of massive numbers of sporozoites. Patent infections can be produced consistently. Drugs or other substances are administered either before or after infection is produced. In the event of prior drug administration, controls are established using identical inocula. When treatment is begun after the infection is established, pre-treatment and post-treatment biopsies are made and the parasites compared with those found in control animals. The infections, both erythrocytic and exoerythrocytic, are followed after treatment to obtain information on relapse.

Parasites affected by the drugs are studied morphologically and histochemically by the application of various staining techniques. Drug levels are obtained when necessary. A flexibility of procedure is maintained so that the methods may be modified to apply to specific problems as they arise.

Major Findings:

Monkeys treated with pyrimethamine prior to infection do not develop patent exoerythrocytic parasites in the liver. Since parasitemia occurs, at least part of the time, in monkeys so treated, parasites must either be surviving at sub-patent levels, surviving in a difficult to detect form, or surviving in tissues other than the liver.

When pyrimethamine treatment is initiated on the sixth day after injection of sporozoites, an effect is evident within 24 hours and very marked after 48. Parasites at 48 hours are pale with foamy cytoplasm. The myriad of nuclei have disappeared, leaving a small number of intensely staining granules which may or may not be related to the nuclei. The parasite after Carnoy fixation shrinks away from the hepatic cell wall more noticeably than do normal parasites. Subsequent to 48 hours of treatment, the parasites become vacuolate, and dissolute. Rather than shrinking on fixation, they show signs of swelling and by 120 hours after treatment, the parasites have become so altered as to be hardly distinguishable. Very little cellular reaction develops, although occasionally mononuclear cells do accumulate around the dying parasites.

Although parasites are not seen in the liver, most animals come down with malaria about 40 days after inoculation, this probably being related to some degree with the persistence of drug in the tissues of the monkey. A working hypothesis is that there is a dormant, non-growing, small stage of the parasite which is not subject to the antifolic type effect of pyrimethamine. These parasites perhaps subsequently develop and produce the parasitemia.

When primaquine is given prior to infection, no parasites are seen in the livers of the monkeys. When primaquine is initiated on the sixth day of treatment, effect is not discernable until 72 to 96 hours after treatment at which time the parasite begins to stain more intensely than normally and dark staining masses appear in the cytoplasm. At 120 hours many parasites appear as dead, amorphous, dark-staining, shrunken masses without any cellular reaction. Studies are now in progress to follow the dying parasites for longer periods.

Both primaquine and pyrimethamine retarded, then stopped the growth of the parasites. This resulted in keeping parasites in the liver in numbers at a time the majority would normally have ruptured and initiated blood infections.

Treatment has been done also using chloroquine, quinine, sulfamethazine, and puromycin. Analysis of the microscopic material is not complete.

Significance to Bio-medical Research and the Program of the Institute:

This project represents another phase of an integrated program of this laboratory and Institute to study the biology and chemotherapy of malaria. The project promises to contribute to an understanding of the basic biological processes of the exoerythrocytic stages of malaria, and to contribute directly to the evaulation of antimalarial drugs. Since primate parasites closely related to those of man are used, the results may apply directly to man. The development of consistent test procedures provides a mechanism, not previously available, for the direct evaluation of drug effects on exoerythrocytic parasites. Since the exoerythrocytic stages are the stages which must be eliminated for radical cure, the studies may have bearing on the use of drugs in malaria eradication.

Proposed Course of Project:

The project shall continue the exploration of established antimalarials with particular emphasis on mode of action. Work on Plasmodium inui shall receive more emphasis as soon as methods for producing consistent exoerythrocytic infections are developed. Reexamination may be made of some antimalarials previously tested

only on blood stages of malaria. Since action on erythrocytic and exoerythrocytic stages is not necessarily closely correlated, there is hope of significant findings. The project shall also determine the effect on the exoerythrocytic stages of such substances as antifolic or folinic acid compounds, anti-purines, amino acid analogs, pantothenic acid analogs, etc.

Part B included: No.

will begin to operate.

The selection of the personnel (ten professional) was guided by the nature of the problem -- availability of space, salaries, and facilities. It was planned to include in the staff workers with experience, who had proved their ability in immunology or in allied fields, and also promising beginners. The latter were included with the hope that they would benefit by working on problems suggested to them or on their own problems. It was hoped that these investigators would soon reach some independence, meanwhile contact with experienced investigators would benefit them. Other workers depending on their interests and experience would work on their problems alone or in small groups. One of the reasons why the idea was favored that the staff should work in small groups or by themselves was that, in general, it is unlikely that persons with independent minds would always be satisfied to be part of a research group. The situation is strikingly different, of course, when the members of the groups represent different disciplines.

Thus far the activities of the staff fall into two larger disciplines; namely immunochemistry and experimental pathology, the latter with special reference to sensitization. The first group is comprised of three members, Drs. Dray, Vannier, and Williams. Up to now their interests have been divergent. Dr. Dray has been engaged in studying isoprecipitinogens in the rabbit; Dr. Vannier has been working on the characterization of certain allergens by physico-chemical, chemical, and serological methods

and, finally, Dr. Williams' studies were concerned mainly with the purification and characterization of a methyl alcohol extract of tubercle bacilli. It has been shown previously that this material contains a substance which immunizes albino mice against infection with virulent tubercle bacilli.

Up to now the immunochemists have been working by themselves; it is hoped, however, that in the near future some of them will be able to cooperate with other staff members engaged in serological studies. This highly desirable plan is expected to be accomplished by the additional space in Building 5 to be available in 1960.

Most of the problems currently studied represent continuation of work begun some years ago. By contrast, a few are new problems or subjects which are being attacked by a conspicuously new method. The most important of these is the introduction of genetically distinct, highly inbred guinea pigs into immunologic research work. The initiation of such studies is of particular importance in the field of delayed type of hypersensitivity. The guinea pig is unique in its suitability to the study of delayed hypersensitiveness. In the guinea pig, high degrees of local (skin, cornea, snout) and systemic reactions (anaphylaxis -- protracted type and delayed type, i.e. tuberculin type) can be produced. In the other experimental animals, these types of reactions may occur only in low degree or not at all. Furthermore, some of these reactions may be difficult to distinguish. For example, the delayed type of skin reaction may be exquisitely intense in the guinea pig and mild in the rabbit and hardly demonstrable in the mouse and in the rat using the same preparatory injections.

It appears now that without the use of highly inbred mice certain problems of transplantable tumors could not be profitably studied. Similarly, the use of two highly inbred families of guinea pigs, No. 13 and No. 2 were demonstrated to be essential for homotransplantation and isotransplantation studies of the skin. The transplantation studies of the skin are only one of very many immunologic subjects to which inbred guinea pigs may be applied. It is very likely that transfer of many kinds of cell born passive sensitization can be achieved using inbred guinea pigs. The chief of the laboratory suggested to Dr. Joseph A. Bauer, Jr. that he carry out experiments to determine whether isotransplantation of the skin within the inbred families and crossing on another would yield the results one would expect. These families have been inbred for about fifty years. The chief of the laboratory's hopes rested on the gross morphologic specimens of tuberculous guinea pigs of families No. 2 and No. 13 which he examined in 1926 at the Henry Phipps Institute of the University of Pennsylvania. These guinea pigs were infected with virulent human tubercle bacilli. When they were killed or died of tuberculosis, the lesions were found to be very similar in the guinea pigs of the same families, while very dissimilar in the animals of family 13 to the lesions found in family 2. There was an interesting relationship found in these guinea pigs with regard to resistance to tuberculosis and antibody formation. Lewis and Loomis observed that tuberculous guinea pigs of family 13 produced antibodies more abundantly to sheep red cells and killed typhoid bacilli than those of family 2.

The experiments on isotransplantation of mouse skin with the aid of inbred families has been successful. Other problems on the transfer of hypersensitiveness to organs are being investigated.

Because of lack of space, a discussion of the reports of the individual investigators is omitted.

Submitted by:

Julius Freund

Julius Freund, M.D., Chief
Laboratory of Immunology, NIAID

Form No. CRP-2
Nov. 1959

PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID - 140
1. Laboratory of Immunology
2. Basic Immunology
3. Bethesda, Maryland

Part A:

Project Title: Studies in the Transplantation of Tissues

Principal Investigator: Dr. Joseph A. Bauer, Jr.

Other Investigators: Dr. Sanford H. Stone, Project Subtitle III, Isologous and Homologous Lymphoid Cell Transplants

Cooperating Units: None

Man Years:

Total: 1.33
Professional: 1
Other: .33

Patient Days: None

Project Description:

Project Subtitle I: The Genetics of Transplantation Immunity in Inbred Guinea Pigs

Objectives:

a. To study the genetic principles governing the transplantation antigens in guinea pigs.

b. To determine the number of histocompatibility genes by which one inbred strain differs from the other.

Methods Employed:

a. Split thickness skin grafts are used as test transplants.

b. The transplant survival in the various donor-recipient relationships of two inbred strains (Strains 2 and 13), their F_1 and F_2 generation hybrids, and backcross animals is examined.

Major Findings:

a. F_1 animals accept both inbred parent strain grafts permanently.

b. F_1 grafts are invariably rejected by either parent strains, but may survive up to twice as long (16 days) as grafts between the two parent strains.

c. The proportion of F_2 animals accepting parent strain grafts suggests a difference in no fewer than two and no more than five histocompatibility genes between the two inbred strains. Backcross grafting results support this assumption.

d. Neither sex chromosomes (X or Y) is associated with histocompatibility antigens.

e. The two inbred strains share strong histocompatibility antigens lacking in Hartley guinea pigs.

Significance to the Program of the Institute:

These studies evaluate the genetics of homograft antigens in two strains of guinea pigs inbred over a fifty year period -- the only guinea pig strains with reported histocompatibility -- which were maintained by the NIH for the past twenty-five years. The inheritance of histocompatibility antigens in accord with orthodox genetic principles suggests a close relationship between antigen and gene.

Proposed Course of Project:

a. A more precise definition of the antigenic differences between the two inbred strains in a statistically adequate number of animals.

b. To elucidate further the mechanisms of prolonged survival of some F_1 to parent strain grafts.

Project Subtitle II: Immunological Tolerance of Homografts in Guinea Pigs

Objectives:

a. To attain permanent survival of an otherwise incompatible homograft by induction of immunological tolerance.

b. To study the nature of the tolerant state and evaluate its dependence on the persistent survival of living cells injected prenatally.

Methods Employed:

a. Guinea pigs at various stages of pregnancy are subjected to laparotomy and fetuses injected directly with viable spleen cell suspensions.

b. Animals treated in this fashion while in utero, are grafted when mature so as to test the presence and degree of tolerance.

Major Findings:

a. Strain 2 guinea pigs injected in utero with Strain 13 spleen cells are tolerant to Strain 13 skin grafts, showing prolonged or permanent survival of grafts which would otherwise be rejected.

b. Albino (Hartley) guinea pigs were similarly made fully or partially tolerant to either inbred Strain (2 or 13). Such albino animals accept pigmented skin grafts (bearing black fur) from the inbred strain donating the prenatal cell injection, but reject grafts from the other inbred strain not used in prenatal injection.

c. Albino guinea pigs injected prenatally with spleen cells of F_1 (Strain 2 x Strain 13 cross) animals are tolerant to F_1 skin grafts; furthermore, these animals also accept skin grafts from both inbred strains (Strain 2 and 13) permanently

d. Spleen cells injected during the middle third of pregnancy (gestation 72 days) usually result in tolerance, whereas injections less than 20 to 25 days prior to birth do not.

Significance to the Program of the Institute:

Extending the induction of experimental homograft tolerance to a new mammalian species (in addition to mice and rats) helps to establish it as a phenomenon potentially applicable to all mammals, possibly including man. The induction of tolerance provides at present the only means of a deliberate and specific prevention of a homograft rejection resulting in acceptance and permanent survival of an otherwise incompatible homograft. In addition to its implications in homotransplantation, the concept of immunological tolerance represents an important stimulus and useful tool for immunologic studies in general and particularly in connection with antibody synthesis, immunologic maturation, autoimmune diseases and delayed hypersensitivity.

Proposed Course of Project:

a. Experiments are in progress to evaluate some of the variables connected with the induction of tolerance.

b. Cells of various genetic content will be used to abolish the induced tolerant state in an effort to evaluate (1) the dependence of tolerance on the survival of cells injected prenatally and (2) the dependence of the state abolished tolerance on the survival of the cells injected for this purpose.

Project Subtitle III: Isologous and Homologous Lymphoid Cell Transplants

Objectives:

a. To compare the relative efficiency of passive transfer of immune reactions within an inbred strain with cell transfers between incompatible guinea pig strains.

b. To study the relationship of homograft immunity induced by transferred lymphoid cells (tested by skin grafts) to the persistence of passively transferred immunity of the immediate and delayed types.

Methods Employed:

a. Inbred guinea pigs are immunized with Mycobacterium paraffin-oil antigen emulsions.

b. Lymphoid cells suspensions are prepared by passage through stainless steel screens.

c. Parallel intraperitoneal injections of cell suspensions into (1) guinea pigs of the same strain and (2) into incompatible guinea pigs (Hartley) are carried out.

d. Cell recipients are tested serially for (1) delayed skin reactivity and (2) serum antibodies.

Major Findings:

a. The passive transfer of tuberculin sensitivity appears to be more effective and of longer duration in intrastrain isologous cell transfers compared to homologous transfers.

b. Homograft immunity to [illegible] lymphoid cells is demonstrable by accelerated skin graft rejections when grafted at three days post cell transfer but much less consistently at four days post transfer.

Significance to the Program of the Institute:

The passive transfer of immune reactions with viable lymphoid cells represents an important tool in the study of a multitude of immune phenomena, particularly in the guinea pig. A priori, cell transfers between histocompatible animals of an inbred strain, should be expected to yield more effective and more representative results as the interfering variable of transplantation immunity to the transferred cells is eliminated, and cells can be expected to survive functionally.

Proposed Course of Project:

a. This project will be continued to confirm and extend these early findings and to explore some of the variables involved.

b. The effect of pre-existent homograft immunity to the transferred cells will be evaluated.

c. Attempts are in progress to produce guinea pig antisera against isoantigenic serum globulin components of one inbred strain. The intended uses of such antisera are (1) as a practical screening test for the identification and differentiation of the two inbred strains and (2) as a test for the functional viability of transplanted lymphoid cells elaborating specifically isoantigenic globulins not present in the recipient.

Part I included Yes ☐ No ☒

Form No. ORP-2
Nov. 1959

PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID - 141
1. Laboratory of Immunology
2. Natural and Acquired Resistance
3. Bethesda, Maryland

Part A:

Project Title: Studies on Antibody Production and Mechanism of Hypersensitivity

Principal Investigator: Dr. Sanford H. Stone

Other Investigators: None

Cooperating Units: None

Man Years:

Total: .66
Professional: .33
Other: .33

Patient Days: None

Project Description:

Objectives:

To study the immunologic, physiologic, and pathologic aspects of protracted anaphylactic shock.

Methods Employed:

Protracted anaphylactic shock caused by injection of homologous antigen in the subcutaneous tissue of sensitized guinea pigs is the experimental model. The mechanism of anaphylactic shock and the role of circulating antibody in anaphylaxis is being studied in actively and passively sensitized guinea pigs of random-bred and inbred strains. Amounts of antibody used to sensitize normal guinea pigs are measured as antibody nitrogen or by using passive cutaneous anaphylaxis techniques.

Major Findings:

Prolongation of anaphylactic shock in guinea pigs is a function of the amount of antigen used in the subcutaneous eliciting dose as well as a function of the circulating antibody. Decreases of antigen dose prolong the syndrome as did increases in amount of antibody. Time of death of the guinea pigs may be taken as a rough measure of total available in vivo antibody. Antigen-antibody complexes formed in vitro will cause lethal anaphylactic shock injected in large amounts into normal guinea pigs. Either the acute or protracted syndromes may be observed. Tests on antihistamine-treated animals

show that organ-bound antibodies rather than circulating antigen-antibody complexes are mainly responsible for the protracted syndrome as well as for the acute anaphylactic shock.

Significance to the Program of the Institute:

Protracted anaphylactic shock in the guinea pig has been known as a rare occurrence and was not studied. Conditions necessary for its demonstration were not well established. It can now be produced in predetermined degrees with regularity. Protracted anaphylaxis in man occurs after subcutaneous or intramuscular injection of allergenic material, and its incidence is higher than that of acute anaphylactic shock with predominantly respiratory symptoms. The mechanism of the acute form, dreaded by clinicians, because treatment may not occur in time, is also clarified by these studies.

Proposed Course of Project:

The combined role of "delayed" type and anaphylactic type sensitivities will be studied in relation to protracted shock syndromes.

Part B included Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 141

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Part B: Honors, Awards, and Publications

Publications:

Stone, S. H.: Acute and Protracted Anaphylactic Shock in Guinea Pigs After Subcutaneous Eliciting Injection of Antigen. J. Immunol. 82:138-145, 1959.

Part A

Project Title: The Application of the Bentonite Flocculation Test (1) to the Diagnosis of Lupus Erythematosus; and (2) to Rabbit Antibodies Against Type I Streptococcal Protein

Principal Investigator: John Bozicevich

Other Investigators: None

Cooperating Units: Drs. John Mason and Donald Kayhoe, Laboratory of Clinical Investigations, Serial No. NIAID - 17C.

Man Years:

Total: 3
Professional: 1
Other: 2

Patient Days: None

Project Description:

Objectives:

1. To apply the bentonite flocculation test to the laboratory diagnosis of lupus erythematosus.

2. To apply the bentonite flocculation test to the detection of Type I streptococcal antibodies produced in rabbits.

Methods Employed:

1. Highly polymerized calf thymus desoxyribonucleic acid (DNA) is dissolved in distilled water and adsorbed onto bentonite particles. A protective colloid, bovine plasma albumin, is added and the suspension dyed with methylene blue. After washing and resuspension of the coated bentonite particles in distilled water, the suspension is used as the material for testing the various sera.

2. Bentonite particles are coated with M-protein material obtained from Streptococcus Type I. After staining and washing, the sensitized particles are used as the test material for the detection of antibodies in rabbit antisera.

Major Findings:

1. The results of the DNA-Bentonite Test with reference to lupus erythematosus are presented in Table I below. It will be noted that the DNA-Bentonite Test compared favorably with the results of the L.E. preparations.

TABLE I

Results of the DNA-Bentonite Test and the Lupus Erythematosus Preparation

Subjects	No.	DNA-Bentonite Test		L.E. Preparation	
		Positive	Negative	Positive	Negative
Lupus (Active)	13	13	0	13	0
Lupus (Remission)	12	3	9	5	7
Rheumatoid Arthritis	64	0	64	8	56
Scleroderma	1	1	0	0	1
Periarteritis Nodosa	1	1	0	0	1
Outpatients and Hospitalized	768	0	768	0	91*
Normal (NIH Employees)	127	0	127	0	10**

2. Acid extract of Type I streptococcal M-protein was prepared. Rabbits injected with this material showed group specificity with respective serological groups when the sera were adsorbed to remove heterologous groups. The flocculation test was approximately 8 times more sensitive than the precipitin test.

Significance to the Program of the Institute:

1. This laboratory aid may assist in the diagnosis of systemic lupus erythematosus. The test is nearly as sensitive as the L.E. preparation and is as specific.

* L.E. preparations performed on 91 cases only.
** L.E. preparations performed on 10 cases only.

2. The streptococcal M-protein flocculation test may be a useful laboratory aid.

Proposed Course of the Project:

To continue studies to improve the sensitivity and specificity of the test for lupus erythematosus. To continue the nucleoprotein aspect of the investigation.

Part B included Yes ☐ No ☒

Part A

Project Title: Characterization of Substances of Bacterial Origin Affecting Resistance to Infection

Principal Investigator: Dr. Curtis A. Williams, Jr.

Other Investigators: None

Cooperating Units: Dr. Rene J. Dubos, Rockefeller Institute

Man Years:

Total: 2
Professional: 1
Other: 1

Patient Days: None

Project Description:

Objectives:

1. Further examination of biologically active materials extracted from tubercle bacilli. Their isolation and composition.

2. To distinguish between the mechanisms of specific and non-specific acquired resistance to infectious diseases.

Methods Employed:

1. Animal protection tests are performed in albino mice. They are studied by bacterial enumeration from infected organs and by survival time.

2. Studies of hypersensitivity and adjuvant activity are carried out in albino guinea pigs.

3. Biologically active substances from tubercle bacilli are compared with bacterial endotoxins from gram negative organisms, which have similar chemical composition and most of the same activities.

4. Chromatography, zone electrophoresis, ultracentrifugation.

Major Findings:

1. Substances derived from methanol extract of tubercle bacilli can be purified. These materials are considered suitable for chemical analysis.

2. Three distinct compounds are of particular interest. One is capable of inducing a moderately durable specific anti-tuberculous resistance in mice. Another, presumed to be chemically similar to the bacterial lipopolysaccharides, increases resistance against a variety of infections. The broad spectrum resistance is rapidly induced but it is of short duration, falling off about three or four weeks after vaccination. The third substance, a "soft wax," in mixture with these other materials augments their effectiveness in conferring resistance against experimental tuberculosis.

3. A model infection produced in mice by a serratia species (tentatively identified as S. plymuthica) is useful for studying the bases of specific and non-specific resistance in mice. In mice there is a very narrow threshold of resistance to these organisms, which can be shifted to higher or lower levels by a variety of treatments.

Significance to the Program of the Institute:

The study of the chemical and immunological nature of fractions from tubercle bacilli is very significant from the standpoint of the production of a prophylactic vaccine against tuberculosis. Information on the fractions of tubercle bacilli may give insight into the pathogenesis and immunity of tuberculosis.

Proposed Course of Project:

Determination of the chemical nature of substances derived from methanol extract of tubercle bacilli which have been demonstrated to play a role in specific and non-specific resistance to experimental tuberculosis.

Part B included Yes [X] No [ ]

Individual Project Report
Calendar Year 1959

Part 3 Honors, Awards and Publications

Publications:

Williams, C. A., Jr., and F. C. Jones: Studies on Fractions of Methanol Extracts of Tubercle Bacilli. I. Fractions which Increase Resistance to Infection. J. Exp. Med., 110:981-1001, 1959.

Williams, C. A., Jr.: Studies on Fractions of Methanol Extracts of Tubercle Bacilli. II. Toxic and Allergenic Properties of Fractions Employed as Antituberculous Vaccines. J. Exp. Med., 111, 1960, in press.

Form No. CRC-2
Rev. 1/59

Serial No. [illegible]
1. Laboratory of
2. Clinical Allergy
3. New York, New

VHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Pathogenesis of Allergic Encephalomyelitis

Principal Investigator: Dr. Philip Y. Paterson

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2
Professional: 1
Other: 1

Patient Days: None

Project Description:

This study of the pathogenesis of allergic encephalomyelitis is carried out at the New York University College of Medicine under [illegible] with the National Institute of Allergy and Infectious Diseases (Ch Serial No. SA-43-PH-1808 and SA-43-PH-3027).

Project Subtitle I: Transfer of Allergic Encephalomyelitis by Mean of Lymph Node Cells

Objectives:

To transfer allergic encephalomyelitis from diseased animals t healthy animals by means of lymph node cells.

Methods Employed:

Adult rats are immunized with rat central nervous tissue emulsi in Freund's complete adjuvant. Lymph node cells, which are known t transfer delayed sensitivity from sensitized to normal animals, are then transferred from these immunized rats to other healthy pregnant recipient rats. These recipient rats are prepared so that they are tolerant to the lymph node cells they receive. Appropriate control are employed.

Major Findings:

By this method of white cell transfer, allergic encephalomyeli has been transferred from immunized donors to non-immunized healthy adult rats, which were made tolerant to the white cells given the As few as 10^7 lymph node cells transferred from immunized donors to tolerant adult recipients results in the appearance of allergic encephalomyelitis in more than 50% of the recipients. By contrast,

the appropriate control animals remain healthy. Furthermore, neonatal recipients of cells from immunized donors remain healthy.

Significance to the Program of the Institute:

Although allergic encephalomyelitis cannot be transferred by immune serum, Freund transferred the disease to normal animals by creating a parabiotic relationship between immunized and non-immunized animals. The successful transfer of allergic encephalomyelitis from diseased to healthy animals by means of lymph node cells alone establishes the role of these cells in the development of this disease and the importance of delayed sensitivity in the pathogenesis of this disease. In addition, the method will prove applicable to the study of certain other diseases.

Proposed Course of the Project:

To continue to study the transfer mechanism to define precisely the exact conditions required for successful transfer.

Project Subtitle II: The Refractoriness of Rats to the Development of Allergic Encephalomyelitis When Exposed to the Antigen Early in Life

Objectives:

To determine the duration of this refractory, tolerant state.

Methods Employed:

Rats, at one day of age, are injected with 100 mgm of guinea pig spinal cord, or with 100 mgm of guinea pig kidney. When these rats are grown to adulthood, they are injected with spinal cord emulsified in Freund's complete adjuvant.

Major Findings:

Of that group of rats injected with guinea pig spinal cord at one day of age, only one-half to one-third develop allergic encephalomyelitis after subsequent injection of guinea pig spinal cord emulsified in Freund's complete adjuvant. The adjuvants were injected when the rats were 10, 16, and 22 weeks old. By contrast, that group of rats injected with guinea pig kidney tissue when one-day of age, uniformly develop allergic encephalomyelitis following the injection of guinea pig spinal cord emulsified in complete adjuvant when the injection is made at the time the rats are 10, 16, or 22 weeks of age.

Significance to the Program of the Institute:

This experiment demonstrates that induction of immunologic refractoriness, or tolerance, can be induced to an antigen which when introduced into an adult may lead to an autoimmune disease.

Proposed Course of Project:

Initial phase of the work is concluded.

Project Subtitle III: Re-induction of Allergic Encephalomyelitis in Rats

Objectives:

To determine whether a relapse of allergic encephalomyelitis can be induced in rats.

Methods Employed:

Adult rats are immunized with spinal cord emulsified in Freund's complete adjuvant. They subsequently develop allergic encephalomyelitis. Those that do not succumb, are allowed to recover. They are then injected again with the antigen and observed for recurrence of the disease.

Major Findings:

These rats did not develop a recurrence of allergic encephalomyelitis when injected a second time with the antigen emulsified in adjuvant.

Significance to the Program of the Institute:

The data suggest a basic difference between the rat and other species in regard to autoimmune tissue damage. This study may provide information concerning desensitization to tissue antigens.

Proposed Course of Project:

To be continued.

Part B included Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 144

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Part B: Honors, Awards, and Publications

Paterson, P. Y.: Organ-Specific Tissue Damage Induced by Mammalian Tissue Adjuvant Emulsions. In: Cellular and Humoral Aspects of the Hypersensitive States (H. S. Lawrence, Editor), Hoeber, New York, 1959, pp 469-503.

Paterson, P. Y.: Tolerance to the Paralytogenic Activity of Nervous Tissue. In: Experimental "Allergic" Encephalomyelitis (M. W. Kies and E. C. Alvord, Jr., Editors), Thomas, Springfield, Ill., 1959, pp 444-450.

Paterson, P. Y.: Designated Discussion, In: Mechanisms of Hypersensitivity (J. H. Shaffer, G. A. LoGrippo, and M. W. Chase, Editors), Little, Brown and Company, Boston, 1959, pp 551-552.

Paterson, P. Y.: Transfer of Allergic Encephalomyelitis in Rats by Means of Lymph Node Cells. J. Exper. Med., 111 (in press).

Part A

Project Title: Studies on the Effectiveness of Glycolipid Fractions of Tubercle Bacilli as Adjuvants in Eliciting Disease States Said to be of the "Delayed" Type of Hypersensitivity

Principal Investigator: Dr. Jules Freund

Other Investigators: Dr. Sanford H. Stone

Cooperating Units: None

Man Years:

Total: 1.0
Professional: .66
Other: .33

Patient Days: None

Project Description:

Objectives:

To study the effectiveness of tuberculo-glycolipid (Wax D) as compared with killed and dried mycobacteria as an adjuvant in inducing allergic encephalomyelitis and aspermatogenesis.

Methods Employed:

Guinea pigs were sensitized by injection of (1) rabbit spinal cord or (2) guinea pig testicular antigens incorporated in complete paraffin oil adjuvants containing decreasing amounts of either tubercle bacilli or the "Wax D" fraction (glycolipid) from tubercle bacilli. The threshold amounts of Wax D or tubercle bacilli necessary for eliciting encephalomyelitis or aspermatogenesis or for sensitization to tuberculin were determined.

Major Findings:

The minimum amount of tubercle bacilli required to induce aspermatogenesis was 0.015 mg, and of Wax D, 0.1 mg; to induce encephalomyelitis, 0.02 mg of tubercle bacilli, 0.2 mg of Wax D. The minimum amount of tubercle bacilli required for sensitization to tuberculin was 0.0004 mg; of Wax D, 0.2 mg. These data indicate that 7-10 times more Wax D than tubercle bacilli was required for inducing allergic encephalomyelitis and aspermatogenesis. Since Wax D is only 6-8% of the whole bacillus, it may be concluded that if Wax D

is the active factor in inducing delayed hypersensitivity, it is 84-160 times more active when contained in the mycobacterium than after isolation and purification. This consideration is not in favor of but does not exclude the role of Wax D in eliciting hypersensitivity of the delayed type.

Significance to the Program of the Institute:

The mechanism of the delayed type of hypersensitivity is not well understood. In man, delayed type of reactivity manifests itself in the tuberculin reaction (both local and systemic) and reactions associated with certain other infectious diseases, including brucellosis, histoplasmosis, and others. In addition, this type of sensitivity leads to both local (skin test) and systemic rejection of tissue or organ grafts, and is responsible for so-called contact eczemas and "poison ivy" type skin reactions. Experiments concerned with the basic mechanism of delayed hypersensitivity may open new routes to control of the phenomenon in man.

Killed mycobacteria present in complete adjuvants "direct" the induction of the delayed type of hypersensitivity to other antigens incorporated in the emulsion. It is evident that a component of the mycobacteria is responsible for this action. It has been claimed that the glycolipid, "Wax D" is the effective material (White, et al). The experiments just described cast doubt upon this claim.

Proposed Course of Project:

This project has been terminated.

Part B included Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 145-A

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Honors:

a. Special Citation, Allergy Foundation of America, July 1959.

b. Albert Lasker Award for 1959, The Albert and Mary Lasker Foundation and The American Public Health Association.

Publications:

Freund, J. and S. H. Stone: The Effectiveness of Tuberculoglycolipid as an Adjuvant in Eliciting Allergic Encephalomyelitis and Aspermatogenesis. J. Immunol. 82:560-567, 1959.

Form No. ORP-2
Nov. 1959

PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID - 146-A
1. Laboratory of Immunology
2. Applied Immunology
3. Bethesda, Maryland

Part A

Project Title: The Resistance of Fetal and Neonatal Gonads to Damage by Maternal Immunization

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1.0
Professional: .50
Other: .50

Patient Days: None

Project Description:

Objectives:

Certain diseases produced by auto-immune reactions are known to depend upon delayed sensitivity. Nevertheless, production of developmental anomalies of the central nervous system and of the lens have been induced in offspring animals by immunization of pregnant females. It was important for further study of auto-immune tissue reactions to find a tissue known to be damaged by delayed auto-sensitivity in the adult, yet insensitive to damage by circulating antibody irrespective of age.

Methods Employed:

Adult female Hartley strain guinea pigs were exposed to adult males of the same strain for a period of one month. After this mating period, the males were removed and the females immunized with testicular tissue emulsified in complete adjuvant. The mothers and offspring were examined for the presence of circulating antibody, and the mothers tested for delayed sensitivity to sperm antigens. The offspring were then scheduled to be sacrificed at various ages, ranging from 2 weeks to 150 days of age, for the purpose of obtaining sections of their tissues for histologic examination with particular reference to their gonads.

Major Findings:

The appearance of those organs obtained to date is normal.

Significance to the Program of the Institute:

This study provides for future experimental work on organ which is known to be damaged by delayed autosensitivity, but which appears totally unreactive to circulating antibody even when exposure occurs at a very early age.

Proposed Course of Project:

This project will be continued until 30 male offspring of immunized mothers have been examined along with an equal number control offspring.

Part B included Yes ☐ No ☒

Form No. CRP-2
Nov. 1959

PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID - 1
1. Laboratory of Immunology
2. Immunochemistry Section
3. Bethesda, Maryland

Part A

Project Title: Immunochemical Studies of the Isoantigens of Serum

Principal Investigator: Dr. Sheldon Dray

Other Investigators: None

Man Years:

Total: .91
Professional: .25
Other: .66

Patient Days: None

Project Description:

Objectives:

a. To identify the antigens in serum which induce isoantibodies

b. To study the chemical nature of these isoantigens.

Methods Employed:

a. Immunochemical analysis by diffusion in agar-gel; simple diffusion in tubes, double diffusion in plates, and immunoelectrophoresis.

b. Passive cutaneous anaphylaxis, tanned-cell hemagglutination and other immunological tests for antibody.

c. Cellulose ion-exchange chromatography and other protein fractionation and purification methods.

d. Zone electrophoresis.

Major Findings:

a. Investigations have shown that components of sera from individual rabbits are antigenic in certain other rabbits and that isoprecipitins can be produced which react with serum antigens having electrophoretic mobilities corresponding to alpha, beta, and gamma globulins.

b. Isoprecipitins use in agar gel immunochemical analysis of 1000 normal sera obtained from several breeds of rabbits showed that the individual rabbits contain one or the other or both of two gamma globulin antigenic specificities in their sera but never lack both of them.

c. A third genetic protein isoantigen in rabbits has been found. The immunochemical relationship of this third antigen to the previous two is under investigation.

d. Additional experiments with sera of patients given several subcutaneous injections of human gamma globulin failed to reveal the formation of iso antibodies.

Significance to the Program of the Institute:

a. Differences in serum proteins between individuals of the same species may be of genetic and clinical significance similar to that of the blood groups. The serum isoantigens may be implicated in some of the unexplained transfusion reactions in man.

b. Serum isoantigens may be useful in the study of "immune tolerance" and perhaps serve as a somewhat analagous experimental model for the study of supposed tolerance to Rh antigen.

c. Isoantigens demonstrated in red blood cells and lymphoid cells do not occur in solution although isoantigens present in red cells have been extracted and are available in pure solution. The serum proteins are the only ones known thus far which occur in solution.

d. Serum protein isoantigens may be particularly suitable for study of some basic problems in immunology concerning the nature of antigen and antibody sites, antigenicity of antibodies, chemical structure of gamma globulin, the antigen-antibody reaction, etc.

Proposed Course of Project:

a. Chemical characterization of the gamma globulin isoantigens in rabbits. Development of methods of assay for each of the isoantigens.

b. Studies on the chemical structure of gamma globulin by papain digestion studies and quantitative assay of antigenic sites.

c. Search for additional gamma globulin isoantigens and additional alpha and beta globulin isoantigens.

d. Specificity of rabbit isoantigens and antigenicity in other species.

e. Isoantigens in other species, particularly mice and man.

f. Isoantigens in other tissues.

Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 147

PHS-NIH
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Part B: Honors, Awards and Publications

Publications:

Dray, Sheldon and Young, G.O.: Two Antigenically Different Gamma Globulins in Domestic Rabbits Revealed by Isoprecipitins. Science 129:1023-1025, 1959

Form No. ORP-2
Nov. 1959

PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID - 147-A
1. Laboratory of Immunology
2. Immunochemistry Section
3. Bethesda, Maryland

Part A

Project Title: Immunochemical Studies on Human Serum Antigens and Antibodies

Principal Investigator: Dr. Sheldon Dray

Other Investigators: None

Man Years:

Total: .91
Professional: .25
Other: .66

Patient Days: None

Project Description:

Objectives:

a. Identification, fractionation, purification, and chemical properties of antigens in human serum.

b. Significance of serum antigens and antibodies in disease.

Methods Employed:

a. Immunochemical analysis by diffusion in agar-gel; simple diffusion in tubes, double diffusion in plates, and immuno-electrophoresis.

b. Cellulose ion-exchange chromatography, zone electrophoresis, and other methods of protein fractionation and purification.

c. Use of primates for immunization with human serum antigens.

Major Findings:

a. Primates were selected for immunization with human serum antigens with the idea that a closely related species to man might yield antibody to components of human serum not readily obtainable in other ways. Results of preliminary experiments with 4 chimpanzees immunized with a single injection of human serum or gamma globulin in complete adjuvant yielded two immune sera to human beta globulin. It seems likely from our results that chimpanzee gamma globulin may be antigenically identical to human gamma globulin. Unfortunately, facilities for further immunization of chimpanzees are not available at present; this work has been discontinued for the present. It is hoped that the study may be resumed in the near future.

b. Immunization of 14 Rhesus monkeys with various antigens from human serum has yielded several relatively strong antisera to many components of human serum (alpha, beta, and gamma globulins). The most striking finding so far has been that of two antigenically different 7S human gamma globulins. These two gamma globulins were present in every normal serum tested to date, but the possibility of gamma globulin groups as found in the rabbits is being explored. Moreover, in two patients with multiple myeloma of the gamma type, we found that one patient had only one of the gamma globulins while the other patient had only the other gamma globulin.

Significance to the Program of the Institute:

a. Should chimpanzee gamma globulin be immunologically compatible in man, immune gamma globulin prepared in chimpanzees may be useful for therapy in connection with infectious diseases where convalescent gamma globulin is not readily available.

b. The ability to distinguish two 7S gamma globulins in humans and to associate the presence of these gamma globulins with multiple myeloma, provides a new approach for studies on the mechanisms involved in this disease.

c. The use of primate antiserum for study of other antigens in serum or antigens in other tissues is a broad problem likely to reveal antigens not previously known.

Proposed Course of the Project:

a. Continue the immunization of primates with various fractions of human serum and to study the antigens which may then be detected.

b. Survey 30 multiple myeloma sera (in collaboration with Dr. John Fahey, Metabolism Branch, NCI) with respect to their reactions with monkey antiserum to normal gamma globulins.

c. Immunization of monkeys with multiple myeloma protein to study the antibodies produced.

d. Study fractions of human serum.

Part B included: Yes ☐ No ☒

Part A

Project Title: Genetic Studies of the Isoantigens in Serum

Principal Investigator: Dr. Sheldon Dray

Other Investigators: None

Man Years: Patient Days: None

Total: .91
Professional: .25
Other: .66

Project Description:

Objectives:

To study the genetic control of serum isoantigens.

Methods Employed:

a. Qualitative tests using primarily agar-gel methods for the identification of isoantigens in families of rabbits and in various rabbit populations.

b. Selective breeding of rabbits to test genetic hypotheses.

Major Findings:

a. Result of immunochemical analysis of sera from 335 offspring of 81 litters are consistent with the hypothesis that the isoantigenic specificity of two gamma globulins, RGG-I and RGG-II, are controlled by a single allelic pair of autosomal genes with both specificities exhibited by the heterozygote. The three genetypes may be designated $\gamma I/\gamma I$, $\gamma II/\gamma II$, and $\gamma I/\gamma II$.

b. As of now, 1006 domestic rabbits of more than 16 breeds have been typed according to their gamma globulin isoantigens; 61 were type RGG-I, 749 were RGG-II, and 196 were RGG-I/RGG-II. The gene frequency varied considerably with the colony tested, only a few colonies showing a significant frequency of the γI gene.

c. The sera of 27 cottontails, 24 jackrabbits, 2 snowshoe hares, and 2 pikas were tested and found to have neither RGG-I nor RGG-II. Thus it appears that these isoantigens are genus specific.

d. A third gamma globulin isoantigen has been found which has been correlated to the presence of RGG-I indicating that RGG-I and RGG-III must be controlled by the same allele or two closely-linked alleles.

e. A fourth gamma globulin isoantigen is under investigation which appears to be controlled by genes at a different locus.

Significance to the program of the Institute:

a. To know the genetic mechanism for the control of isoantigens is essential for the usefulness of these isoantigens in studies of basic immunology and hypersensitivity. The molecules having antigenic sites under the genetic control postulated are antibody proteins, namely, gamma globulins. Some of these molecules may also react as antibodies. As antigens, precipitable by anti-sera, they should be subject to quantitative estimation. Moreover these molecules may be expected to pass through maternal-fetal barriers. Thus, this immunochemical genetic system may be uniquely suited for studies of some basic problems in immunology, hypersensitivity, embryology, and protein chemistry.

Proposed Course of Project:

a. To establish a source of rabbits of the three known gamma-globulin types so that they may be available for other studies.

b. To study the newer factors of new gamma-globulin isoantigens. The preliminary finding that alleles at more than one locus control gamma-globulin isoantigenic sites has encouraged further genetic studies.

Part B included Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 147-B

PHS-NIH
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Part B: Honors, Awards, and Publications

Publications:

Dray, Sheldon, and Young, Glendowlyn O. Genetic Control of Two Gamma-Globulin Isoantigenic Sites in Domestic Rabbits. Science, in press.

Form No. ORP-2
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PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID - 147-C
1. Laboratory of Immunology
2. Immunochemistry Section
3. Bethesda, Maryland

Part A

Project Title: Application of Serum Isoantigen-Isoantibody Systems to Studies in Basic Immunology and Hypersensitivity

Principal Investigator: Dr. Sheldon Dray

Other Investigators: None

Man Years:

Total: .91
Professional: .25
Other: .66

Patient Days: None

Project Description:

Objectives:

a. To apply the serum-isoantigen-isoantibody system to problems in basic immunology and hypersensitivity.

b. The first such study has been to investigate the question as to whether the isoantigens would be useful in the study of so-called "immune tolerance" or "immunologic unresponsiveness."

Methods Employed:

a. Selective breeding of rabbits on the basis of known genetics of gamma-globulin isoantigens.

b. Immunochemical methods to evaluate production of isoantibodies and presence of isoantigens.

c. Quantitative assay of isoantigens by agar-gel methods.

Major Findings:

a. Results of preliminary experiments suggest that "immunologic unresponsiveness" to gamma-globulin isoantigens may be induced in rabbit offspring in the following ways. The offspring may be exposed to the "foreign" isoantigen naturally during pregnancy as a result of the "foreign" isoantigen of the mother passing through the maternal-fetal barrier. The offspring may also be exposed artificially by transfusion of the mother with the "foreign" isoantigen during the last week of pregnancy.

b. The duration of "immunologic unresponsiveness" is at least seven or eight months; none of the offspring under investigation have as yet produced detectable isoantibodies.

Significance to the Program of the Institute:

"Immunologic unresponsiveness" has been one of the most interesting problems in hypersensitivity during the last few years as a result of new developments in our knowledge of tissue grafting. Should the isoantigen-isoantibody system be effective in the induction of "tolerance," this system may very well be most suited for a study of some of the basic mechanisms involved in "tolerance." The isoantigen-isoantibody system has the advantage that one may work with soluble proteins (rather than tissue) and with the precipitating antibodies (rather than tissue rejection).

Proposed Course of Project:

a. Continue the study of "immunologic unresponsiveness."

b. Compare antibody production in rabbits of different gamma-globulin types.

c. Study the biological effects of isoantigen-isoantibody reactions in vivo: Serum sickness, Arthus reactions, etc.

d. Synthesis of gamma globulin in the fetus and newborn.

e. Use of isoantigens as cell markers in studies of delayed hypersensitivity.

Part B included Yes ☐ No ☒

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PHS-NIH
Individual Project Report
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Serial No. NIAID - 148
1. Laboratory of Immunology
2. Applied Immunology
3. Bethesda, Maryland

Part A

Project Title: Basic Studies on the Cellular Localization of Antigens, Antibodies, and Other Substances by the Fluorescent Antibody Technique and Fluorescence Microscopy

Principal Investigator: Dr. John E. Tobie

Other Investigators: Dr. Jules Freund, Project Subtitle I, Antibody Formation in Neonatal Rabbits

Cooperating Units:

Project Subtitle II - Drs. Robert A. Milch and R. A. Robinson, Department of Orthopedic Surgery, Johns Hopkins University Hospital

Project Subtitle III - Dr. Henry K. Beye, LCI, Serial No. NIAID - 190

Project Subtitle IV - Dr. G. Robert Coatney, LPC, Serial No. NIAID - 130

Project Subtitle V - Mrs. Eleanor J. Tobie, LPD, Serial No. NIAID - 123D

Man Years:

Total: 3.25
Professional: 1.25
Other: 2.0

Patient Days: None

Project Description:

Project Subtitle I: Antibody Formation in Neonatal Rabbits

Objectives:

To perform studies on the types of cells involved in antibody formation and to determine the capacity of neonatal animals to synthesize antibody.

Methods Employed:

Neonatal and adult rabbits have been given a single injection of bovine serum albumen, crystalline egg albumin or killed typhoid organisms into the footpad. The animals were bled frequently and checked either by the sensitive Boyden hemagglutination or agglutination test in a effort to determine the earliest time at which circulating antibodies first appear. In certain animals lymph nodes were removed and the node fluid tested for presence of antibody.

Major Findings:

Neonatal animals given a single injection of antigen, without the addition of adjuvants, failed to produce antibodies. Lymph nodes were not examined. The following results refer to animals which were injected with antigens combined with adjuvants. Frequent antibody titrations on the neonatal animals injected once showed that in some of the rabbits, antibodies in the blood plasma became demonstrable in less than 1 month, while in some only after 2 or 3 months. Subsequently, the titers rose as in primary immunization. One of the interesting findings was that in a few animals 6 to 10 weeks after injection, the draining lymph nodes were large and cystic; and the fluid per ml contained more antibodies than the serum. In several animals no antibody, as determined by the Boyden Hemagglutination Test, could be demonstrated in the serum in spite of the presence of antibodies in the cystic fluid of the lymph node. When some of the rabbits, several weeks after the first injection, received a second injection of the antigen, they gave a characteristic primary and other rabbits a characteristic secondary response.

Significance to the Program of the Institute:

A study of the nature of the antibody response in neonatal animals may lead to a better understanding of the reasons for immunological immaturity in the infant. The influence of age upon antibody formation is an important factor in the capacity of infants to be actively immunized.

Proposed Course of Project:

Because the formation of antibodies in neonatal rabbits as compared with adults is very slow, there seems to be a better opportunity to study the cellular aspects of antibody formation in the neonatal animal. Therefore, the studies will be continued along these lines.

Project Subtitle II: Localization of the Tetracyclines in Bone Formations Associated with Certain Tumors

Objectives:

To determine the specific sites of deposition of the tetracycline antibiotics in the abnormal bone formation of various tumors.

Methods Employed:

Tumors were obtained immediately after their excision from patients who had previously been administered tetracycline. The specimens were quick-frozen and thin fresh-frozen sections of the tumors were cut in a cryostat. Localization of the fluorescent tetracycline in the bone formations was accomplished by fluorescence microscopy.

Major Findings:

The microscopic localization of the tetracyclines has been discovered in the bone formations associated with a variety of tumors

including osteogenic sarcoma, chondrosarcoma, and osteochondroma. The tetracyclines were deposited in newly proliferated and not in old well-established bone. It was observed that in areas where the calcium salts recently had been layed down, the tetracyclines were deposited. It is suggested that the deposition of the tetracycline in bone arises from a complex interfacial interaction which involves, presumably, the four-ringed naphthacenecarboxamide nucleus of the drugs, calcium and the organic matrix of newly proliferated bone.

Significance to the Program of the Institute:

The precise localization of the tetracyclines in the bone formations associated with certain malignant tumors would seem to offer therapeutic possibilities. The selective deposition of these antibiotics also provides an excellent means whereby histological evidence of specific biological processes in bone may be obtained.

Proposed Course of Project:

The studies on the localization of the tetracyclines in the abnormal bone of tumors have been concluded and the results are in preparation for publication.

Project Subtitle III: Localization of Filarial Worms by Fluorescent Methods

Objectives:

To devise a diagnostic method whereby subcutaneous filarial worms can be localized by fluorescent methods after the administration of tetracycline and to determine the cellular localization of the drug within the worms.

Methods Employed:

A patient with subcutaneous migrating filarial worms (probably Loa loa) was given tetracycline. Twenty-four hours later the skin of the patient was stimulated with ultra-violet light and the worms localized by their specific yellow fluorescence. A dog infected with the heart worm Dirofilaria immitis was given tetracycline. Samples of blood were taken and examined at fluorescence microscopy to determine the uptake of the antibiotic by the microfilariae. The animal was sacrificed, the adult worms recovered and fresh-frozen sections prepared. Localization of the drug in the worms was accomplished at fluorescence microscopy.

Major Findings:

A patient with subcutaneous migrating filarial worms (probably Loa loa) was administered tetracycline and the worms subsequently localized

by stimulating the skin with ultraviolet light. The worms, as demonstrated by their yellow fluorescence under the skin, were observed to move from one location to another over a period of hours. It was apparent that the worms selectively took up more tetracycline than the surrounding tissues. In an effort to determine the localization of the drug within an adult filarial worm, a dog infected with Dirofilaria immitis was administered tetracycline. The adult worms were recovered, fresh-frozen sections prepared and by fluorescent methods the drug found to be localized throughout most of the internal organs of the worms including the uterus and its contained embryos. There was no drug in the cuticle of the worm. Also, it was demonstrated that the drug was deposited selectively in the microbilariae of D. immitis taken from peripheral blood.

Significance to the Program of the Institute:

The ability to localize migrating subcutaneous filarial worms by a simple fluorescent method, may lead to a practical diagnostic method. The selective deposition of the tetracyclines in filarial worms opens up the possibility of preparing certain drug complexes for therapeutic trials.

Proposed Course of Project:

The experiments on the localization of filarial worms in human subjects by fluorescent methods will be expanded to include other worm infections.

Project Subtitle IV: Fluorescent Antibody Studies on Malaria Parasites

Objectives:

To specifically stain malaria parasites, by fluorescent antibody methods, for the rapid examination of smears by an automatic scanning microscope.

Methods Employed:

Antiserum against Plasmodium berghei will be produced and its globulin fraction labeled with fluorescein isothiocyanate. After the fluorescent antibody is applied to blood smears, the specific fluorescence of the malaria parasites will be observed in the fluorescence microscope. If the experiments are successful, the fluorescent antibody technique will be applied to human malaria preparations. With the aid of an automatic microscope, equipped with a photoelectric cell, rapid scanning of slides in large scale malaria surveys may be carried out.

Major Findings:

Preliminary investigations on dehemoglobinized thin and thick films of Plasmodium berghei observed under darkfield, visible light illumina-

tion, have demonstrated that the malaria parasites are conspicuously and distinctly visible. In order to obtain malaria parasites free from mouse red cells, antibodies against mouse red cells in high titer have been produced in rabbits. By themselves these findings are not of major significance, but in view of the significance of the project of which they are a part, they seem to be worth mentioning.

Significance to the Program of the Institute:

The fluorescent antibody studies on malaria could lead to a method whereby hundreds of malaria smears could be scanned in a single day. This would be of great importancein connection with the program of worldwide malaria eradication.

Proposed Course of Project:

The fluorescent antibody studies on malaria will be continued along the lines outlined.

Project Subtitle V: Localization of Trypanosoma rangeli in the Insect Host

Objectives:

To determine the organ localization of Trypanosoma rangeli in the insect host.

Methods Employed:

Fresh-frozen sections of the triatomes, which serve as the insect host of Trypanosoma rangeli, are being prepared and the organ localization of the trypanosomes accomplished during different stages of its cycle in the insect.

Major Findings:

In fresh-frozen sections of the insect host, Rhodnius prolixus, the flagellate Trypansoma rangeli has been localized in the salivary glands.

Significance to the Program of the Institute:

The successful localization of Trypanosoma rangeli in fresh, frozen sections of the insect host opens up the possibility of the precise localization of other infectious agents in arthropods.

Proposed Course of Project:

The localization of Trypanosoma rangeli in the insect host will be studied during the different stages of its cycle in the arthropod.

Part B included Yes ☐ No ☒

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Individual Project Report
Calendar Year 1959

Serial No. NIAID - 149
1. Laboratory of Immunology
2. Natural and Acquired Resistance
3. Bethesda, Maryland

Part A:

Project Title: Studies on a New Manifestation of "Delayed" Type Hypersensitivity: Hemorrhagic Reactions in Guinea Pigs Sensitized to Ovalbumin

Principal Investigator: Dr. Sanford H. Stone

Other Investigators: Dr. Jules Freund

Cooperating Units: Clinical Pathology Department, Hematology Service, Dr. George Brecher

Man Years:

Total: 1.16
Professional: .83
Other: .33

Patient Days: None

Project Description:

Objectives:

To study the mechanism and significance of a new manifestation of "delayed" hypersensitivity, namely the hemorrhagic reaction in guinea pigs sensitized with paraffin oil adjuvants containing mycobacteria.

Methods Employed:

Guinea pigs actively sensitized by injecting egg albumin incorporated in paraffin oil adjuvants containing Mycobacterium tuberculosis var. hominis (Strain "Jamaica 22"). The massive hemorrhage occurring at the site of subcutaneous injection of large amounts (50 mg) of homologous antigen in sensitized guinea pigs serves as the experimental model. The effects of varying the dose of mycobacteria, site of sensitizing injection, strain of guinea pig, and amount of antigen in the eliciting dose were studied. Furthermore, the hematological changes (white blood cell counts, platelet counts, hematocrit, and hemoglobin determinations) and the histological observations of the hemorrhagic reactions were described.

Major Findings:

The massive hemorrhages in the skin and subcutaneous tissue of guinea pigs sensitized with egg albumin incorporated in water-in-oil adjuvants containing mycobacteria failed to occur in guinea pigs sensitized by the injection of egg albumin in oil at one site and

the mycobacteria in oil at a remote site. This indicates that the presence of killed tubercle bacilli at the site of injection of antigen is essential for the modification of the process of sensitization manifested by hemorrhage and links this manifestation to reactivity of the "delayed" type, i.e. hypersensitivity associated with the white cells rather than the serum. Hartley guinea pigs (random bred) were more susceptible than Strain 13 and Strain 2 guinea pigs and required in the order of 50 times less mycobacteria for minimal sensitization. Females are more susceptible than males.

Systemic reactions occur after subcutaneous challenge in guinea pigs sensitized to egg albumin with or without mycobacteria. There is a sharp decrease in numbers of platelets in both groups of animals, so that the hemorrhage seen only in those sensitized using mycobacteria involves an additional factor which is probably inherent in the condition of "delayed" sensitivity. Histologic sections from hemorrhagic sites are clearly distinguishable from those from animals which were sensitized without mycobacteria. The latter resemble sections from classic Arthus reactions while the hemorrhagic sites showed mainly extravasated red blood cells in very large amounts and disrupted collagen fibers.

Significance to the Program of the Institute:

The delayed type of hypersensitivity to soluble proteins is not easy to demonstrate, especially in animals which have circulating antibodies. The hemorrhagic reaction, which is clearly different from Arthus reactions on a gross and histological level, becomes a tool for the study of the "delayed" type of reactivity in animals which have or do not have concomitant "immediate" type of sensitivity. The significance and mechanism of "delayed" type hypersensitivity may be explored. Furthermore, a relationship between the hemorrhagic reaction, the Schwartzman reaction, and certain obscure hemorrhagic phenomena seen in man is indicated, and may be investigated using this experimental mode.

Proposed Course of Project:

The significance of the hemorrhagic reaction as a manifestation of "delayed" type hypersensitivity will be further explored.

Part B included Yes [X] No [ ]

(Attachment I)

Serial No. NIAID - 149

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Part B: Honors, Awards, and Publications

Publications:

Stone, S. H. and Freund, J.: Hemorrhagic Reactions in Animals Sensitized With Ovalbumin in Adjuvants Containing Mycobacteria. II. Relation to "Delayed" Hypersensitivity. Proc. Soc. Exp. Biol. Med. 102:140-146, 1959.

[illegible]
1 [illegible]
2 Applied Immunology
3 Bethesda, Maryland

Part A

Project Title: The Relationship of Delayed and Immediate Hypersensitivity to Allergic Thyroiditis

Principal Investigator: Dr. Philip R. B. McMaster

Other Investigators: None

Cooperating Units: None

Man Years: Patient Days: None

Total: 1.0
Professional: .50
Other: .50

Project Description:

Objectives:

To determine the relative importance of delayed and immediate sensitivity to thyroid extract in the production of allergic thyroiditis.

Methods Employed:

Two groups of strain 13 guinea pigs, one immunized with the aid of complete adjuvant, the other with the aid of incomplete adjuvant, will be compared. The development of circulating antibodies, delayed sensitivity, and allergic thyroiditis in these two groups of animals will be followed. Particular emphasis will be placed upon the relationship of delayed sensitivity to the development of the disease. Once this aspect has been completed, attempts to transfer this disease from immune to non-immune animals will be made with leukocyte transfers, and compared with the effect of similar attempts to transfer the disease with antisera.

Major Findings:

Several small groups of animals have been injected with the antigen emulsified in complete or incomplete adjuvant. The antigenic dosage has been varied to determine the most effective dose. The appearance of delayed sensitivity in these animals has been correlated with the development of the disease.

Significance of the Project to the Institute:

This project may establish the importance of delayed sensitivity in the development of allergic thyroiditis.

Proposed Course of the Project:

It is proposed to continue the work at the maximum antigenic stimulus to establish whether or not there is a real correlation between the development of delayed sensitivity and the development of the disease.

Part A

Project Title: Fractionation and Specific Purification of Antibody

Principal Investigator: Dr. Wilson E. Vannier

Other Investigators: None

Cooperating Units:

1. The portion of this work involving the specific purification and fractionation of anti arsanilic acid antibody by the use of solid adsorbents (arsanilic acid coupled to cellulose) is being carried out in joint collaboration with Dr. Jean-Marie Dubert of the Pasteur Institute, presently a visiting research worker in Professor Ray Owen's immunology group of the Division of Biology, California Institute of Technology.

2. The specific adsorbent used in this study and the methods for its preparation were developed in our immunochemistry group of the California Institute of Technology by Dr. William P. Bryan presently at the Carlsberg Laboratory in Denmark.

Man Years: Patient Days: None

Total: .83
Professional: .33
Other: .50

Project Description.

Objectives:

1. To explore possible specific methods of antibody purification.

2. To achieve the chromatographic fractionation of specifically purified antibody and study changes in antibody specific bonding affinity during the course of immunization.

3. To study the chemical and physical properties of "heavy" (S19) antibodies.

Methods Employed:

1. Specific antigen antibody precipitates are dissociated in [illegible] at pH 2.7 and the antibody separated from the antigen by [illegible] electrophoresis in the same buffer.

2. The use of p-arsanilic acid coupled cellulose as a specific adsorbent for anti-hapten antibody is being explored.

3. We plan to study the possibility of the use of the hapten-cellulose adsorbent for column chromatography of antibodies. From an equilibrium dialysis study of the fractions from the column, it is hoped that a distribution curve showing the relative amount of antibody of differing affinity for hapten may be obtained. In this way it should be possible to study the heterogeneity of antibody with respect to affinity for a specific hapten as a function of the degree and method of immunization.

4. The Wassermann reagin antibody-lipid antigen system is being used to study the properties of rapidly sedimenting antibodies, since methods are already available for specific purification and syphilitic serum can be obtained in fairly large amounts. The methods of starch block electrophoresis, DEAE cellulose chromatography and preparative ultracentrifugation are being used to isolate the antibody. After specifically purified rapidly sedimenting antibody has been obtained, we plan to study its physical and chemical properties, and the effect of change of pH, the effect of -SH compounds such as mercaptoethanol and other reagents on these properties and the reactivity for antigen.

Major Findings:

1. Purified antibody fractions have been obtained, using the BSA rabbit antiBSA system and the starch block electrophoresis method; however, the yield or purity of the antibody has not been determined.

2. It has been demonstrated that antibody can be adsorbed on the arsanilic acid coupled cellulose and removed by low pH or hapten solutions; however, the quantitative aspects of this process have not been explored.

3. We are investigating the possibility of carrying out a separation of the S19 serum proteins from whole serum by the use of DEAE cellulose chromatography. A definite method has not been perfected as yet.

Significance to the Program of the Institute:

The general problem of specific purification of antibody is one that is of basic importance for chemical work in immunology. The presence of large amounts of nonspecific protein with the antibody complicates or prevents a study of the chemical reactions of antibodies, the structure of antibodies, or the physical properties of antibodies. A study of the heterogeneity of antibodies with regard to affinity for a specific known antigenic determinant and especially the influence of degree or route of immunization on the distribution of antibodies found will provide additional basic information about the nature of the immune response. It is possible that such information could be correlated with ideas about the mechanisms of antibody synthesis.

Proposed Course of Project:

This project is in its earlymost stages and the general plan to be followed has been indicated above under other headings so that the few things that have been done would be understandable.

Part B included: Yes ☐ No ☒

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Nov. 1959

PHS-NIH
Individual Project Report
Calendar Year 1959

Serial No. NIAID - 153
1. Laboratory of Immunology
2. Clinical Allergy
3. Pasadena, California

Part A

Project Title: Basic Studies on the Chemical, Physical, and Skin Reactive Properties of Extracts of House Dust

Principal Investigator: Dr. Wilton E. Vannier

Other Investigators: None

Cooperating Units:

Dr. Ernest M. Heimlich of the Department of Pediatrics of the University of California at Los Angeles and Dr. A. M. Targow, a practicing allergist in Los Angeles are testing various house dust fractions for skin reactivity in specifically sensitive individuals.

Man Years:

Total: .83
Professional: 0.6
Other: .0.7

Patient Days: None

Project Description:

Objectives:

1. To study the chemical and physical properties of the materials used clinically for the diagnosis and treatment of house dust allergy.

2. To try to identify the components responsible for the specific skin reactivity.

Methods Employed:

1. Fractions of aqueous house dust extracts have been prepared and studied by chemical and physical methods and evaluated for activity by direct skin test.

2. Studies are in progress to determine the effect of chemical or enzymatic modification of the dust fractions on the specific skin reactivity.

Major Findings:

1. It has been found that the dust allergen fractions consist of a heterogeneous mixture of acidic polysaccharides with 5-20% polypeptide (as described in Induvidual Project Report for 1958). During the period covered by this report the starch block electrophoresis

study previously described has been extended to include two commercial dust allergen preparations (Hollister-Stier Laboratories and Endo Laboratories, Inc.). The composition of these materials is similar to that of our purified fraction. The starch block electrophoresis experiments carried out at low pH (ca. 1.8) and in 1.7M urea (pH 2.5) indicate that the polypeptide and polysaccharide portions of the materials are linked through covalent bonds rather than being present as separate components or linked by ionic bonds or multiple hydrogen bonds.

2. The studies involving chemical or enzymatic modification of the dust fractions have not proceeded far enough to yield any major findings.

Significance to the Program of the Institute:

1. About one-third of all allergic individuals give a positive skin reaction to house dust extracts. These extracts have been used for many years in the diagnosis and treatment of house dust allergy. It is of importance to investigate the chemical nature of these materials and to find out what chemical structures are involved in the skin reactions.

2. A rational standardization of the potency of house dust allergen extracts might well be based on a knowledge of the chemical nature of the active materials.

Proposed Course of the Project:

The study of chemically modified and enzyme treated dust fractions will be continued.

Part P included Yes [X] No [ ]

(Attachment 7)

Serial No. NIAID 153

PHS-NIH
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Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications:

Vannier, Wilton E. and Campbell, Dan H.: The Isolation and Characterization of a Purified House Dust Allergen Fraction. J. Allergy 30:198-218, 1959.

Part A

Project Title: Ultracentrifuge Studies of Skin Sensitizing Antibody in Allergy

Principal Investigator: Dr. Wilton E. Vannier

Other Investigators: None

Cooperating Units:

This work is being carried out in collaboration with Dr. Ernest M. Heimlich of the Department of Pediatrics of the University of California at Los Angeles.

Man Years:

Total: .33
Professional: .33
Other: 0

Patient Days: None

Project Description:

Objectives:

To study the sedimentation properties of the skin sensitizing antibodies of the sera of individuals with atopic allergies.

Methods Employed:

1. Sera from allergic individuals were fractionated by sedimentation in the preparative ultracentrifuge and in the Waugh partition cell in the analytical ultracentrifuge. The fractions obtained have been characterized by analytical ultracentrifugation, electrophoresis, and passive transfer skin reaction (P. K. Test).

2. Recently studies involving sedimentation in salt density gradients have been initiated.

Major Findings:

1. As previously reported (Individual Project Report for 1958), our data show that the skin sensitizing activity cannot be exclusively associated with the S19 component and that probably the S19 component is not active at all. Our data and more recent experiments carried out by Dr. Sehon and Dr. Julius Gordon at Montreal are all consistent with the hypothesis that the skin sensitizing activity is associated with a serum component of intermediate sedimentation coefficient, i.e. between 7S and 18S.

2. The only new finding is that the skin sensitizing antibody appears to be associated with serum proteins of hydrated density of about 1.36 rather than being present in the lipoprotein fractions of density 1.2 or lower.

Significance to the Program of the Institute:

The physical properties of the skin sensitizing antibody of atopic allergy are of fundamental importance in understanding allergic reactions. The skin sensitizing antibody is qualitatively different from most ordinary precipitating antibody in its electrophoretic and sedimentation properties and in that it is retained locally when injected intradermally rather than diffusing away. The process of antibody fixation to tissues is an important problem in all allergic reactions and any differences that can be demonstrated in the chemical or physical properties between ordinary precipitating and skin sensitizing antibody may be important in this process.

Proposed Course of Project:

The experiments involving density gradient centrifugation studies will be continued.

Part B included Yes ☐ No ☒

MEMORANDUM

Rocky Mountain Laboratory
Hamilton, Montana
December 11, 1959

To: Dr. Dorland J. Davis
Associate Director, NIAID
National Insitutes of Health
Bethesda 14, Maryland

From: Carl L. Larson, Director C.L.L.

Subject: Summary of Research Progress, Rocky Mountain Laboratory, 1959

The research program at the Rocky Mountain Laboratory has continued to expand and to be productive during the past year. Possibly the most significant expansion has resulted from renewed interest in Q fever occasioned by our findings that this disease is present and spreading among dairy cattle in the United States and that a large number of cases of severe illness have occurred in persons residing in Twin Falls County, Idaho. Concomitant with discovery of human cases, serologic surveys of certain population groups in Idaho were performed revealing the presence of a large number of persons who had specific antibodies against Coxiella burnetii even though it was not possible to elicit evidence of clinical illness. Epidemiologic investigation of persons engaged in the dairy trade in the Twin Falls area conducted by Dr. Stoenner revealed that those associated with infected herds had more evidence of illness than those whose herds were free of Q fever. Similar studies are now being done in Ada County, Idaho, an area where only serologic evidence of disease has been encountered. It is hoped that these studies will throw some light upon the source of infection and upon the reason for the obvious difference in reaction to exposure shown by individuals in the two areas.

The situation in Montana can be illustrated best by quoting figures collected by Dr. Lucto. Among 8,000 dairy herds in the state there are 119 containing infected animals. Most infections are concentrated in the milk sheds and only near Kalispell are herds found to be free. In Ravalli County, the herd infection rate is 4.2%; in Gallatin County and Teton County the rate is 7.0% and 10.0%. Among 11,277 human serums examined, 162 or 1.4% were found to have antibodies against C. burnetii. While these figures indicate that Q fever is a problem in Montana, data accumulated on the spread of infection in dairy cattle are more enlightening. Dairy cattle from selected dairy sheds were surveyed for the presence of infection in February 1959 and 45 of 1,741 herds were found to be infected. Similar surveys conducted in October 1959 showed 119 of these herds to be infected. In 8 months the herd infection rate had increased from 2.6% to 6.8%.

The discrepancy between the number of clinical human cases, serologically positive persons, and the presence of a vast animal reservoir of infection will be investigated. Although only a very few clinical cases have occurred in Montana, it has been found that these persons have been associated with cattle from which it has been possible to isolate organisms of about the same degree of virulence encountered previously in California. The questions of whether or not relatively avirulent strains exist in cattle, whether or not antibodies present in infected milk influence the course of human disease, or whether some other animal such as sheep are factors in determining human reaction are being studied. In Ravalli County, studies have been initiated to determine if and how human disease may develop during a period when the disease is spreading among dairy cattle.

In addition to these epidemiologic investigations, attempts are being made to develop suitable immunization procedures for cattle, sheep, and man. No previous studies of specific prophylaxis of Q fever in sheep have been made. In cattle, a vaccine made of living Brucella abortus (strain 19) diluted in heat-killed suspensions of C. burnetii is being employed. Situations are being surveyed where it would be practical to immunize human beings and to determine if, by eliminating all individuals with positive serologic reactions and skin-test reactions, it would be possible to eliminate any untoward reactions to vaccine.

Experience with interpretation of serologic tests of human serums has emphasized the need for inclusion of both Phase I and Phase II organisms in separate complement-fixation tests before conclusions can be drawn. By virtue of performing CF tests in this way, many discrepancies formerly noted between the results of CA tests and of skin tests can be eliminated.

Laboratory investigations have been directed largely toward production of C. burnetii on modified Zinsser tissue cultures, purification of these organisms, and extraction of rickettsiae to obtain antigens. C. burnetii can be extracted with dimethyl sulfoxide, and a component acting as a haptene in the CF system has been obtained quantitatively by Dr. Ormsbee. When C. burnetii is grown on tissue cultures by Mr. Pickens, there is an increase in the number of organisms and in the neighborhood of 1 to 2.5 mg of rickettsiae can be obtained from each gram of tissue. By judicious application of continuous sucrose gradients, it is possible to purify the organisms so that no contaminating material can be observed by electron microscopy. Recent results from Drs. Ribi and Hoyer show glycerol-water gradients to be equally as valuable in this purification process. Since the entire procedure is one which has been adapted to relatively large-scale experimental methods and is one in which chick tissue need not be employed, it is obvious

that it has application to many problems dealing with immunity and sensitivity in Q fever. The problem of sensitivity will be dealt with in the very near future.

Dr. E. J. Bell has devoted considerable time to studies of the various factors of importance in preparation of vaccines for protection against Q fever and Rocky Mountain spotted fever. In the first instance there is considerable need for a standard product which may be used in our laboratory investigations and in the latter there is need for a better preparation which may be used to protect individuals liable to exposure. He has also demonstrated the value of toxin-neutralization tests in identifying the species of rickettsiae contained in the spotted fever group and has emphasized the differences which may occur. In addition, field work has pointed up the differences in virulence in isolates of R. rickettsii from ticks collected in areas where the severity of spotted fever in man varies considerably.

One of the very interesting observations coming from such field investigations is that the incidence of ticks infected with rickettsiae in areas where many ticks are found to harbor CTF virus is practically nil. Results of preliminary experimental studies indicate that ticks which have been infected as larvae or nymphs with CTF virus prior to or after infection with R. rickettsiae yield only CTF virus when tested for both organisms after they have moulted to the adult stage.

In further studies of the ecology, Drs. Burgdorfer and Eklund have been able to re-emphasize the fact that microecological conditions greatly influence the incidence of infection of rodents and ticks with CTF virus. In Montana, at least, the presence of golden-mantled ground squirrels greatly facilitates the maintenance and spread of virus. Studies were made of the duration and height of viremia in native rodents, and it was found that viremia in this rodent is both prolonged and of sufficient severity to account for infection of ticks feeding upon them.

Drs. Eklund and Thomas have had considerable success in producing an antigen from brains of mice infected with CTF virus which is effectively killed and still is capable of producing neutralizing antibodies in recipient animals. In previous studies in which virus was inactivated with 1:4,000 formaldehyde at 37° C, they were unable to demonstrate antigenicity in the product. Present preparations are inactivated at 4° C for periods as long as 60 days before all virus is killed. Dr. Meiklejohn at the University of Colorado is testing these vaccines in humans. It is interesting to note that with the old vaccines there was evidence in human beings that while the occurrence of disease was not prevented, the clinical illness appeared to be considerably less severe than in nonimmunized

persons. That this disease is by no means an uncommon one in the West is demonstrated by isolation of virus from 553 patients to date and by the observation that 8% and 3% of serums from persons living in rural areas of Wyoming and Idaho possess neutralizing antibodies against CTF virus.

Considerable interest has been manifested in the strain of virus isolated in Powassan, Ontario, by Dr. McLean. A field investigation by Mr. Kohls was most valuable in de-emphasizing the idea that this virus might be transmitted by ticks, since few ticks are present in the area and the periods during which cases occur do not coincide with periods when ticks are active. Laboratory studies likewise have shown this virus to have, at best, only a remote serologic relation to the viruses of Russian spring-summer encephalitis and of louping ill. It is of interest that serums from residents of rural areas of the West and Alaska were all negative when tested for antibodies against this virus.

Because of recent isolations of viruses in addition to inornata and trivittatus virus from mosquitoes, Drs. Eklund and Thomas have increased the number of arbor viruses available for laboratory study, and serologic studies are being expanded in order to enable them to identify agents that are encountered. They are also developing methods for classifying these viruses on a biological basis, using such criteria as host range of infectivity and degree of viremia produced in diverse hosts in order to obtain information which could be helpful in field investigations.

The presence of rabies in bats has continued to be a problem. This year isolations were made from 11 bats, representative of 3 genera and 4 species, including Eptesicus fuscus, Myotis lucifugus, M. evotis, and Lasionycteris noctivagans. Three isolations were made directly in mice following bat bites. It is regrettable that the cooperative project contemplated with the University of Montana did not materialize since it is of extreme importance that population studies be instituted in order to obtain valid information on the importance of bats as a potential source of infection for man and animals and to establish bat colonies in the laboratory so that experimental studies are not limited in time. While attempts will be made to continue to do these things, progress will be severely hampered by lack of support of the proposed cooperative program. The presence of Histoplasma capsulatum in bats was noted.

Studies of tularemia were limited to those concerning immunity in laboratory animals and to studies of virulence of strains of Bacterium tularense isolated in various parts of the world. There has also been considerable interest in the problem of whether or not B. tularense can be transmitted transovarially by the tick Dermacentor andersoni. Preliminary results obtained by Drs. Bell

and Burgdorfer, using animal inoculation and fluorescent microscopy for identification of infection in female ticks and eggs, indicate that the organism is present in the germinal follicles but not in the egg when viewed in situ or in eggs tested by injection into susceptible animals.

A great deal of interest has been manifested by a number of investigators in the techniques Dr. Ribi has developed for the physical isolation of cell walls and protoplasmic fractions of various organisms. Isolation of these fractions makes it much simpler to collect and purify antigenic materials useful for diagnostic or immunological purposes. In conjunction with Dr. Munoz, of the University of Montana, investigations were made of the role of cell walls and protoplasm of Bordetella pertussis in producing certain effects in mice. It had been postulated previously that cell walls of this organism might confer protective immunity to mice, but no definite proof of this had been obtained. Our experiments showed that protoplasm contained a heat-labile toxin which was toxic both for mice and for the skin of rabbits and that the cell walls contained the histamine-sensitizing factor and the protective antigen. Since this problem was brought to us by Dr. Munoz, no definite plans are made to continue it, but Drs. Devlin and Stimpert, of Parke, Davis & Co., have been interested in the results and have visited the Laboratory in order to learn the technique. They plan to apply the method to commercial production of whooping cough vaccine with the idea of seeing whether or not a vaccine which will pass the NIH standards can be manufactured.

Dr. Gerhardt, of the University of Wisconsin, has followed the development of this research program closely and has submitted spores of two species of bacteria (Bacillus subtilis and B. terminalis) for disintegration and isolation of various morphologic elements. The electron microscope photographs included will demonstrate the excellent results obtained. It will be noted that preliminary rupture of the organism results in the isolation of the spore itself and of the exosporium coat and that treatment of the spores by ultrasonic vibration results in isolation of the spore proper and of spore walls. These fractions are to be used by Dr. Gerhardt and his co-workers in certain immunologic and chemical studies.

In conjunction with Dr. Foster, of the University of Alabama, studies were made of the components of Brucella abortus to determine their toxicity and their potentialities as protective antigens. Cell wall and protoplasmic fractions were obtained and a water-soluble material was obtained by the extraction of whole cells with ether. When studied in mice, it was found that the protective index conferred by these various materials was as follows: controls = 0, protoplasm = 1.0, acetone-dried whole cells = 1.9, residue ether extract = 2.7, cell walls = 3.7, and aqueous ether

extract = >4.6. The cell walls and the aqueous ether extract are potent endotoxins for mice. The antigen extracted from Br. abortus by ether is soluble in water and is toxic. It resembles similar antigens obtained from Salmonella in these respects, but differs from that of Bacterium tularense since in the latter organism this treatment results in the isolation of a particulate nontoxic fraction.

Thus, the studies carried out this year serve to extend observations made in the past which emphasize the importance of cell wall fractions and of the cell wall itself in producing protective immunity in experimental animals. This upholds the theory that protective antigens of bacteria should be located upon the surface of the organisms rather than in the interior. Studies with Salmonella have been directed mainly toward determining the effect of lipid which is contained in the so-called complete antigens. According to Westphal, the lipid contained in this protein-carbohydrate-lipid complex is both toxic and immunogenic. This idea is difficult to accept in view of the evidence accumulated that carbohydrates and proteins are in general responsible for immunogenic reactions.

Studies carried out at the Rocky Mountain Laboratory and in conjunction with Dr. Landy, of the Cancer Institute, show that the lipid fraction of complete antigens is not responsible for many of the biological activities of complete antigens of Salmonella. By reducing the lipid content of antigens from approximately 19% to 1% or 2% and testing the original and the delipified antigen by various methods, it was found that the delipified material reacted, by and large, in the same way as the normal antigen. Thus, they were toxic, they produced pyrogenic reactions in rabbits, they produced skin lesions in rabbits, they had a tumor-damaging factor, they were toxic for thorotrast-treated rabbits, and both conferred specific and nonspecific protection upon mice which were immunized with them. It was found that lipid did not produce specific protection in mice, but was capable of inducing nonspecific resistance.

Our original enthusiasm for concentrating efforts to obtain cell wall fractions of tubercle bacilli to immunize animals against tuberculous infection has been dampened considerably by the consistently negative results obtained in immunized mice challenged by the pulmonary route. While it has been possible to show that killed antigens are capable of protecting mice against infections produced by intravenous inoculation of virulent organisms, it has not been possible to demonstrate that cell walls, protoplasm, or phenol-killed antigens are capable of producing significant immunity in mice which are subsequently challenged with a relatively small dose of virulent organisms given by aerosol. On the other hand, preparations of living R37 or H37Ra will effectively immunize mice against aerosol infection, and the dose of avirulent organisms

given as vaccine may be considerably diluted and still confer protective immunity. It has also been demonstrated that significant immunity persists for at least 26 weeks after administration of vaccine. One of the interesting aspects of this study has been that the results in mice are similar to those obtained in human populations where it is stated that the incidence of pulmonary tuberculosis is not greatly affected by vaccination with BCG, but the incidence of tuberculous meningitis and miliary tuberculosis and other secondary syndromes is greatly decreased. In our experimental system it can be shown that bacteremia is uncommon following infection of immunized mice, whereas organisms are found consistently in the lungs even in the absence of tubercles. As a basis for a potency test for living vaccines to be used in man, this system appears to be quantitative and to result in a graded immunity response in mice.

With cell walls obtained from Mycobacterium butyricum and M. tuberculosis, it has been possible to demonstrate the specificity of the hypersensitivity produced in rabbits by injection of one or the other of these fractions. There is considerable need for this type of test and, in conjunction with serological studies, it may be possible to demonstrate chemical and antigenic differences in various species and strains of mycobacteria.

Drs. Bell and Ribi have found that cell walls of acid-fast bacilli potentiate the immune response of guinea pigs toward brain antigens contained in Freund's adjuvant. Protoplasm is needed in concentrations about 100-fold greater than cell walls in order to produce similar iso-allergic responses.

Investigations continued by Drs. Salvin and Smith serve to add emphasis to the theory that delayed hypersensitivity is a preliminary and essential step in production of immediate sensitivity and circulating antibodies. This relation of delayed to immediate hypersensitivity was strengthened by demonstration of the anamnestic response displayed by animals receiving a primary dose of antigen so small that they develop only delayed hypersensitivity but develop circulating antibodies very rapidly if a secondary stimulus is applied at the time delayed hypersensitivity is at its height. Since delayed hypersensitivity to a protein-haptene complex is directed toward the broad protein portion of the moiety and immediate hypersensitivity toward the specific haptene portion, it is evident that specificity of the immune reaction in animals changes as the process matures. These data showing that during the process of developing immunity there is a broad area toward which antigens are directed suggest that administration of one type of poliovirus (type I) may, very early, produce immunity to heterologous types (II and III) which may be accelerated and increased by secondary injections of the heterologous types of poliovirus.

Antigens of Trichophyton rubrum are being employed to study the role of hypersensitivity in skin infections due to this organism or to T. mentagrophytes. Patients suffering from infections with the latter organism display delayed reactions to the antigen, but patients infected with T. rubrum fail to do so, suggesting that the reason for the development of chronic infections with T. rubrum is due to its lack of antigens which are responsible for production of immunity. Studies of the pathogenesis of histoplasmosis in normal and immunized dogs challenged by the pulmonary route are under way.

Studies of poliovirus by Dr. Hoyer continue to be exciting. From a practical point of view, the discovery of methods whereby large quantities of infected tissue-culture fluid can be concentrated and virus purified makes it feasible to produce purified virus for immunogenic studies. Routine harvests of 5 X 10" PFU of virus are obtained. Based on 10^6 PFU as a suitable immunizing dose for man, about 500,000 doses of vaccine is produced with our limited experimental facilities. From a theoretical viewpoint, his data indicating that the RNA of poliovirus is made up of a single strand which can be inactivated by disintegration of a single bond are fundamental to studies of proliferation of animal viruses. Stress is placed upon observations of the failure of S^{35} and P^{32} of virus used to infect cells to be incorporated in significant amounts into new virus. These observations differ from those made regarding tobacco mosaic virus and bacteriophage. It should be noted that labeling of poliovirus with S^{35} contained in methionine serves to mark the protein faction of the virus in contrast to the marking of nucleic acid which is accomplished with P^{32}. Labeling of protein of poliovirus by this method has not been reported previously.

Dr. Philip has continued his studies of tick paralysis and has established that hamsters, marmots, dogs, foxes, and monkeys develop disease when female ticks are allowed to feed upon them. An interesting observation which may have some relation to the transmission of tick paralysis is the appearance of a previously undescribed fat-like body developing in female ticks during engorgement. In conjunction with Dr. Esplin, of the University of Utah, investigations of the pharmacological action of the toxin have been expanded, and it is now definite that the central as well as the peripheral nervous system is involved in this disease.

Mr. Kohls and Dr. Brennan have continued their ecological and taxonomic studies of ticks and mites. It is evident that the collections of these arthropods maintained at the Rocky Mountain Laboratory are among the best in the world, and advice is sought by scientists interested in problems connected with diseases transmitted by these "insects." Progress in the taxonomy of mites has progressed to the point where it is scientifically possible

to begin investigations to determine their real and potential importance as vectors of agents responsible for disease in man and animals. A project is being planned as a cooperative effort between MARU and RML. This promises to be an exciting study which should yield some highly interesting results.

An important side-product of our fluorescent antibody studies of disease agents in ticks is the discovery that serial sections of ticks can be easily made by sectioning them in the cryostat. Sections are obtained in which the various organs are not disturbed. These sections are suitable for studies of normal and abnormal histology in a way which was not possible previously.

During the past year, programs concerned with Q fever and insect-borne diseases have been particularly emphasized. As we previously pointed out, there is a considerable reservoir of infection in animals in the western United States, and this makes it apparent that sooner or later human disease will occur and present a considerable public health problem. At the present time, however, we have not encountered a great deal of human illness, but our program is set up to care for possible situations which may arise. In order to augment this program, Dr. Michael Gregg and Dr. Kenneth Gerner have been added to the staff.

With recognition that our program of medical entomology should receive added support, we are in the process of hiring two entomologists who are trained in the fields of acarology. It is hoped that a program will shortly be operating in Panama in cooperation with MARU and it has been suggested that a similar program might be carried out in cooperation with Dr. Hoogstraal of NAMRU 3 in Cairo, Egypt. Projects of this type are especially valuable since they remove any provincial taint from our entomological research and properly allow us to study important insect-borne diseases in their natural environment.

Although considerable emphasis is being given to field investigations and insect- and animal-borne diseases, it is still the opinion of the director of the Rocky Mountain Laboratory that work being done on more basic aspects of microbiology, such as the role of cell walls in immunity, the life cycle of poliovirus, and the correlation of delayed hypersensitivity to immediate hypersensitivity are problems of fundamental importance and deserve to be supported to the utmost.

During this year we have considerably increased our production of scientific publications. Last year we pointed out that we had published 35 papers and that 29 others had been accepted by journals. This was an increase of 25% compared to 1957. This year we had 49 published papers and 29 accepted by journals, an increase of 23%. We feel these papers represent a fair distribution of our scientific production.

It might be of interest to point out that we have had 47 professional visitors during the last year and of these 11 were from foreign countries. Many of these individuals spent a sufficiently long time at the Rocky Mountain Laboratory to perform or initiate research projects. Our project on the growth of Q fever organisms in modified Zinsser tissue cultures was initiated through a visit from Dr. Jacob Gaon, of Yugoslavia.

Previous annual reports have pointed out the financial difficulty in operating the Rocky Mountain Laboratory on the usually accepted financial division of 80% of allotted funds for personal services and the balance for other expenses. While this problem still exists, it has not been so acute during the last and the current fiscal years. This has been due largely to the facts that considerable support from other than station-allotted funds has been received in the form of equipment purchases and that vacancies in our personnel ceiling have gone unfilled. It is felt that there would be a definite financial problem at present if there were not from fifteen to twenty positions vacant. Equipment purchases have been held to a minimum for some time now and, as a result, there is a backlog of requests totaling from $20,000 to $30,000. In addition, we are faced with the expense of equipping and occupying the new animal building.

ROCKY MOUNTAIN LABORATORY, HAMILTON, MONTANA

ANNUAL REPORT, 1959

NIAID-170 OFFICE OF THE DIRECTOR
C. I. Larson

NIAID-171 RICKETTSIAL INFECTIONS
Principal Investigator: C. B. Philip
Others: E. J. Bell, G. M. Kohls, D. B. Lackman, R. A. Ormsbee, H. G. Stoenner

NIAID-172 Q FEVER
Principal Investigators: L. Luoto, H. G. Stoenner
Others: K. A. Berne, M. B. Gregg, B. H. Hoyer, D. B. Lackman, R. A. Ormsbee, E. Ribi

NIAID-173 DISEASES HAVING A RESERVOIR OF INFECTION IN THE NATURAL ENVIRONMENT
Principal Investigator: J. F. Bell
Others: W. L. Jellison, D. B. Lackman, M. T. McKee, C. R. Owen, H. G. Stoenner

NIAID-174 TRANSMISSION OF DISEASE AGENTS BY CERTAIN VECTORS
Principal Investigator: C. B. Philip
Others: J. F. Bell, J. M. Brennan, W. Burgdorfer, W. L. Jellison, G. M. Kohls, D. B. Lackman

NIAID-175 ALLERGY AND IMMUNOLOGY OF FUNGAL INFECTIONS AND THE MECHANISMS OF ALLERGIC PHENOMENA
Principal Investigator: S. B. Salvin
Other: E. F. Smith

NIAID-176 VIRUSES, VIRUS COMPONENTS, AND VIRUS SURFACES
Principal Investigator: B. H. Hoyer
Others: R. K. Berloff, F. G. Jarvis, R. A. Ormsbee, D. B. Ritter

NIAID-177 IMMUNE PROPHYLAXIS OF MYCOBACTERIAL INFECTIONS
Principal Investigator: E. Ribi
Others: W. T. Haskins, C. L. Larson

NIAID-178 IMMUNE PROPHYLAXIS OF SALMONELLA INFECTIONS
Principal investigator: E. Ribi
Others: W. T. Haskins, F. G. Jarvis, K. C. Milner

NIAID-179 INVESTIGATIONS OF THE ROLE OF MORPHOLOGICAL ELEMENTS OF MICROORGANISMS IN IMMUNITY AND RELATED PHENOMENA
Principal investigator: E. Ribi
Others: B. L. Larson, K. C. Milner

NIAID-180 THE ENCEPHALITIDES
Principal investigator: C. M. Eklund
Others: W. Burgdorfer, W. L. Jellison, G. M. Kohls, D. B. Lackman, L. A. Thomas

NIAID-181 COLORADO TICK FEVER
Principal investigator: C. M. Eklund
Others: W. Burgdorfer, W. L. Jellison, G. M. Kohls, D. B. Lackman, L. A. Thomas

Serial No. NIAID-170
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Office of the Director

Principal Investigator: C. L. Larson

Other Investigators: None

Cooperating Units:

Man Years (calendar year 1959):
Total: 30 (Includes 10 vacant positions not other-
Professional: 1 wise allocated to a project)
Other: 29

Project Description:

The over-all direction and supervision of the scientific research program of the Rocky Mountain Laboratory; the direction of the administrative aspects of the Laboratory including all fiscal, supply, housekeeping, and maintenance activities; and the providing of scientific services as required by the research staff, including laboratory animal care and breeding, sterile glassware and media preparation, special shop services, library, graphic arts, etc.

Part B included Yes X No ____

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Eklund, Carl M., Hadlow, William J., Pickens, Edgar G., and Gerloff, Robert K.: Detecting poliovirus in vaccine lots used in Idaho in 1955. Pub. Health Rep. 74(1): 60-66. Jan. 1959.

Gerloff, Robert K., Larson, Carl L., and Glesne, Lillian R. B.: Naturally occurring poliomyelitis antibody in Hamilton, Montana. Pub. Health Rep. 74(2): 175-180. Feb. 1959.

Lackman, David, Casey, Mary, Philip, Robert, Owen, Cora, and Reinhard, Karl: A comparison of influenza in the northwestern United States caused by A-prime and Asian influenza viruses. Can. J. Pub. Health 50(2): 71-79. Feb. 1959.

Philip, R. N., Reinhard, K. R., and Lackman, D. B.: Observations of a mumps epidemic in a "virgin" population. Am. J. Hyg. 69(2): 91-111. Mar. 1959.

Philip, Robert N., Weeks, Willard T., Reinhard, Karl R., Lackman, David B., and French, Carolyn: Observations on Asian influenza on two Alaskan Islands. Pub. Health Rep. 74(8): 737-745. Aug. 1959.

Collins, A. Patrick: Library of the Rocky Mountain Laboratory. Stechert-Hafner Book News, Stechert-Hafner, Inc. & Hafner Publ. Co. New York. 14(1): 1-2. Sept. 1959.

In press:

Lackman, David, Gerloff, Robert, and Philip, Robert: A comparison of complement fixation and metabolism inhibition as methods of evaluating poliomyelitis antibody response. Am. J. Pub. Health.

Serial No. NIAID-171
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Rickettsial Infections

Principal Investigator: C. B. Philip

Other Investigators: E. J. Bell, G. M. Kohls, D. B. Lackman, R. A. Ormsbee, and H. G. Stoenner

Cooperating Units:

Man Years (calendar year 1959):
Total: 10.75
Professional: 2.75
Other: 8

Project Description:

Objectives:

These studies are directed toward obtaining a better knowledge of diseases caused by rickettsiae. During this year most of the studies were concerned with the spotted fever group of organisms, and attention was given to classification and differentiation of the rickettsiae in this group, toward development of a better vaccine, and toward studies of the natural history of spotted fever.

Methods:

The methods employed are largely the standard ones used in studies of rickettsial diseases. During the last few years, however, a toxin has been discovered to be a specific entity which is characteristic of certain species of rickettsiae and consequently is employed in the differentiation of these organisms.

Major findings:

1. By employing the mouse toxicity test, it has been shown that the rickettsia responsible for Siberian tick typhus is more closely related to that which has been isolated from Amblyomma

Part B included Yes X No ___

maculatum than to R. rickettsii and R. conorii. There is also a partial cross relationship between R. conorii and the rickettsiae associated with Siberian tick typhus or isolated from A. maculatum.

2. The problem of production of spotted fever vaccine has been considered primarily because commercial producers have difficulty in manufacturing consistently effective batches of vaccine. It has been shown that vaccine loses potency during storage. It has also been found that if 7-day embryos are used for the production of rickettsiae rather than 4- to 6-day-old embryos, which are ordinarily used, more organisms can be harvested and thus more potent vaccine produced. Animals immunized with vaccines develop antitoxins at levels consistent with the potency of vaccine. It is suggested that the toxin-neutralization test be used for the evaluation and standardization of vaccines rather than the cumbersome immunization test.

3. Human beings who develop spotted fever in Idaho are less seriously affected that those who contract the disease in Montana. It has been assumed that the strains of rickettsia present in Idaho are less virulent than those in Montana, but until now no experimental support has been available for this idea. Isolations made from ticks in Idaho show that the rickettsiae are indeed less virulent, for they produce fewer days of fever, a shorter duration of scrotal swelling, and that they fail to produce scrotal necrosis or mortality in guinea pigs into which they are injected. They do, however, produce toxins.

Significance:

While the need for spotted fever vaccine is not as great as it has been in the past, there is still considerable demand for this product. Therefore, the demonstration that better vaccines can be produced and that better methods are available for standardization is of significance to commercial manufacturers and to that segment of the population which desires to be immunized. Since tick-borne diseases constitute a public health menace in many parts of the United States, it is necessary to discover as many facts as possible about the methods whereby disease-producing agents are maintained in nature and to determine how the virulence of such organisms varies from area to area. The demonstration that rickettsiae from an area such as Idaho where spotted fever cases are known to be relatively mild are less virulent for experimental animals than those from areas where human cases are serious emphasizes the importance of the influence of local ecological features upon the incidence and severity of certain human diseases.

Proposed course:

It is planned to continue studies of conditions necessary for the production of spotted fever vaccine to the point at which

it will be possible to provide data useful in revising regulations for manufacture of this product. Studies of toxins of various species and groups of rickettsiae will be continued and expanded in order to utilize their characteristics for classification of the organisms and for an understanding of the pathogenesis of the various diseases for which they are responsible.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Philip, Cornelius B.: Some epidemiological considerations in Rocky Mountain spotted fever. Pub. Health Rep. 74(7): 595-600. July 1959.

Philip, Cornelius B., Hughes, Lyndahl E., Rao, K. N. A., and Kalra, S. Lal: Studies on Indian tick typhus and its relation to other human, typhuslike rickettsioses. Arquivos V Congresso Internacional de Microbiologia, Rio de Janeiro, Aug. 17-24, 1950. 1: 571-574. 1958.

In press:

Bell, E. John and Stoenner, Herbert G.: Immunologic relationships among the spotted fever group of rickettsias determined by toxin neutralization tests in mice with convalescent animal serums. J. Immunol.

Serial No. NIAID-172
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Q fever

Principal Investigators: L. Lucto and H. G. Stoenner

Other Investigators: K. A. Gerner, M. B. Gregg, B. H. Hoyer, D. B. Lackman, R. A. Ormsbee, and E. Ribi

Cooperating Units:

Man Years (calendar year 1959):
Total: 16.25
Professional: 6.25
Other: 10

Project Description:

Objectives:

Q fever has been a vexing public health problem and one which, for a variety of reasons, has not received the attention it has deserved. Our present studies have two objectives. The first of these is to understand the methods whereby Coxiella burnetii is spread from host to host, the role of certain animals in producing infections in man, the extent and degree of infection in human beings, and the discovery and application of methods of control of disease in animals and man. The second is to develop laboratory methods directed toward the diagnosis and prevention of this disease and others caused by related rickettsiae. Included in this area are attempts to grow rickettsiae on tissue cultures and defined media, development of methods to purify organisms grown on tissue cultures or embryonated eggs, and isolation of fractions useful for diagnostic and prophylactic purposes.

Because of the variety of findings, it is deemed best to consider this project in two categories: "A" deals primarily with field investigations and "B" with laboratory studies.

Part B included Yes X No ___

Methods:

The methods here employed are primarily those of clinical and epidemiologic approaches to infectious disease. Field investigations and serologic tests are performed.

Major findings:

1. In a survey of cattle involving a sample of 400,000 cattle in 27,000 herds in 39 of 50 states, Q fever was found in each state. The disease was discovered in both Hawaii and Alaska. Evidence was collected indicating that bovine infection is spreading in at least 13 states.

2. 119 infected dairy herds have been detected in Montana. The areas where Q fever exists are focal in nature and are associated with the dairy industry. One important factor in the spread of the disease among cattle is the sale and dispersal of infected herds.

3. In Montana 1.4% of 11,277 human serums and in Idaho 1.9% of 12,413 human serums contained antibodies against C. burnetii.

4. Strains of C. burnetii isolated in Montana from cattle on farms where cases of Q fever in human beings occurred were found to be equally as virulent for experimental animals as were strains previously isolated in California.

5. In Twin Falls County, Idaho, where a number of human cases of Q fever have occurred, a study was made of 50 families associated with infected herds of dairy cattle and 48 associated with noninfected herds. Among 134 persons in the former group 32 had antibodies against C. burnetii, and among the 134 in the latter group only 18 had antibodies. Twenty-three of 36 persons working with infected cattle were skin-test positive, while 10 of 24 persons dealing with noninfected cattle had positive skin tests. Clinical illness compatible with Q fever was more commonly associated with persons with specific antibodies.

Significance:

The presence of a large and increasing reservoir of infection of Q fever in cattle throughout the United States and the presence in cattle of strains of C. burnetii of sufficient virulence to produce disease in man have been demonstrated. While many of the factors responsible for the spread of disease from animals to man are not fully understood, it is obvious that infected domestic animals constitute a public health menace. There is evidence that a large number of inapparent or unrecognized cases of Q fever occur in the human population, but the epidemiologic significance of persons with antibodies or sensitivity to C. burnetii is not known

at present. With Q fever now widespread in the United States and animal infections increasing in numbers and concentration, an opportunity is presented to study the factors responsible for the transmission of Q fever to man and to attempt to apply general and specific methods of control.

Proposed course:

Further studies will be made to detect and study clinical cases of Q fever in man and to isolate organisms from such cases. Studies have been initiated near Boise, Idaho, (an area where there is a high incidence of individuals with antibodies against C. burnetii but only few clinical cases of Q fever) to determine the differences between persons associated with infected and noninfected herds of dairy cattle. A study is being started in Ravalli County, Montana, to observe the spread of disease among bovines and the resultant spread, if any, to persons whose occupation brings them into contact with cattle. Attempts will also be made in both Idaho and Montana to study the impact of ovine infections upon cattle and man.

Studies, in cooperation with the Montana State Board of Health and the Montana Livestock Sanitary Bureau, also have been started to determine if vaccination will control infection in sheep and to determine if it is possible to immunize cattle against Q fever and undulant fever with a single preparation containing viable bacteria and killed rickettsiae.

Studies have been initiated to evaluate methods available for the diagnosis of Q fever in man. Within the scope of this investigation are problems raised by discovery and utilization of a skin test and the question of the relative importance of Phase I and II antibodies.

B. Laboratory Studies:

Methods:

Modified Zinnser tissue cultures are prepared and utilized for growth of C. burnetii, and organisms grown in this media and in embryonated eggs are subjected to chromatography or centrifugation in sugar gradients to isolate and purify them. Attempts are being made to determine the antigenic make-up of these organisms by employing certain organic solvents to extract the antigens.

Major findings:

1. C. burnetii has been grown on embryonic mouse, guinea pig, and chicken tissues maintained on a base containing

serum and agar in addition to certain salts. Viable organisms may be obtained from such cultures for at least 100 days after inoculation. Proliferation of organisms appears to be greatest during the first 10 days after the cultures are infected. Production of rickettsiae is comparable to that obtained with embryonated eggs.

2. By centrifugation of suspensions of yolk sacs or tissue cultures containing rickettsia in M/1 KCl followed by centrifugation in a linear sucrose gradient, it has been possible to obtain purified rickettsial suspensions which by electron microscopic examination are free of contaminating particulate material. The yield of C. burnetii is in the range of 1.0 to 2.5 mg per gram of yolk sac or tissue culture. Phase I and II organisms may be purified by the sucrose-gradient method, and Phase I rickettsia may also be concentrated by centrifugation in M/1 KCl. Phase II organisms have not been purified by the latter method.

3. Dimethyl sulfoxide will extract specific complement-fixing antigen from C. burnetii. The antigen is soluble in ether but not in water. It does not induce sensitivity when injected intradermally into rabbits and only feebly elicits delayed reactions in sensitized animals. The antigen does not produce protective immunity in recipient hosts, but treatment of organisms with DMS does not harm the residual protective antigen.

4. A skin test for the detection of individuals displaying hypersensitivity to products of C. burnetii has been developed.

5. The factors of importance in standardization of Q fever vaccines have been investigated, and methods of evaluating potency have been analyzed.

Significance:

Adequate numbers of rickettsiae for experimental and production purposes previously have been obtainable only by growing organisms on embryonated eggs. With tissue culture methods, chromatography, and gradient centrifugation, large quantities of C. burnetii in a pure state may be obtained which may be employed for a variety of studies. Studies of the metabolism of rickettsia may be more profitably pursued with these preparations, and attempts to isolate immunogenic and allergenic portions of the organisms will be facilitated.

The finding that complement-fixing antigens may be obtained from C. burnetii without interfering with the immunogenic activity of the remainder of the cell indicates that it may be feasible to extract further antigens that will effectively produce protective immunity. These findings may have a particular bearing upon skin-test procedures. Application of a skin test to detect individuals

sensitive to products of this organism has considerable importance for epidemiologic surveys of Q fever and for mass immunization procedures. A knowledge of the antigenic fractions will be needed before the proper skin-test material may be selected.

Proposed course:

It is planned to continue studies on tissue culture procedures in order to work out a practical method of producing rickettsiae in mass quantities and in order to determine the cell and nutrient requirements for adequate growth. By continuation of studies to purify rickettsiae and to isolate antigens from such purified preparations, it should be possible to obtain antigens which are effective in a variety of ways such as in complement fixation, skin tests, and the production of protective immunity. Cell walls and protoplasm also will be studied for their activity in these systems and especially for their role in the hypersensitive state which has been demonstrated.

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Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Lucto, Lauri: The epidemiology of Q fever in the United States. Am. J. Pub. Health 49(3): 334-338. Mar. 1959.

Stoenner, Herbert G., Jellison, William L., Lackman, David B., Brock, Darrell, and Casey, Mary: Q fever in Idaho. Am. J. Hyg. 69(3): 202-213. May 1959.

Stoenner, Herbert G., Holdenried, Robert, Lackman, David, and Orsborn, John S., Jr.: The occurrence of Coxiella burnetii, Brucella, and other pathogens among fauna of the Great Salt Lake desert in Utah. Am. J. Trop. Med. & Hyg. 8(5): 590-596. Sept. 1959.

In press:

Lackman, David B., Bell, J. Frederick, Larson, Carl L., Casey, Mary L., and Benson, W. W.: An intradermal sensitivity test for Q fever in man. Arch. Pasteur Inst., Tunis.

Stoenner, Herbert G. and Lackman, David B.: The biologic properties of Coxiella burnetii isolated from rodents collected in Utah. Am. J. Hyg.

Luoto, Lauri: The nation-wide occurrence of Q fever infections in cattle. Pub. Health Rep.

Serial No. NIAID-173
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Fiscal Year 1959

Part A.

Project Title. Diseases having a reservoir of infection in the natural environment.

Principal Investigator. J. F. Bell

Other Investigators: W. L. Jellison, D. B. Lackman, M. T. McKee, G. R. Owen, and H. G. Stoenner

Cooperating Units:

Man Years (calendar year 1959):
Total: 11
Professional: 4
Other: 7

Project Description:

Objectives:

Studies performed in this project are directed toward an understanding of the ecology of those diseases transmissible to man from animals and the natural environment. As a direct continuation of the ecological studies, attention is paid to the laboratory aspects of the problems encountered with special emphasis on diagnosis, treatment, and control.

Methods:

The methods employed are, in general, straightforward microbiological techniques, with those variations called for by the material under consideration.

Major findings:

1. Rabies virus has been isolated from 11 bats. The virus was present in the brains of all 11, in the salivary glands of 6, and in the brown fat of 8. The virus was also isolated from oral swabs of 3 bats and from white mice that were bitten by these same animals. Virus was isolated from representatives of 3 genera and 4 species of bats, namely, Eptesicus fuscus, Myotis lucifugus, Myotis evotis, and Lasionycteris noctivagans.

Part B included Yes X No ____

2. It has been demonstrated that white mice may recover from infections with virulent street rabies virus, developing paralytic sequelae which result in stunting or dwarfing of mice infected as juveniles and among larger animals, an intense pruritis, giving rise to severe excoriation and even amputations. As a further sequela of the paralysis heavy louse infestations result.

3. The pulmonary fungus, Emmonsia, has been demonstrated in rodents in Norway, Sweden, and Denmark. This fungus, when used to infect animals such as dogs, sheep, and monkeys, produces a severe and fatal disease. In dogs it has been possible to demonstrate the presence of antibodies by the precipitin test and the presence of delayed hypersensitive reactions to products isolated from the spores of this organism.

4. Studies of the virulence of strains of Bacterium tularense from Russia, France, and the United States indicate that the organisms from the Old World are approximately as virulent as those of the United States associated with water and aquatic animals. Previously, it was believed that greater virulence of strains present in the Old World might account for the higher incidence of tularemia in Europe, especially in Russia.

5. Studies on immunity against tularemic infections have been continued. It has been shown that an Ascoli type antigen is capable of protecting both mice and guinea pigs against infection with a moderately virulent strain of B. tularense. Studies were initiated to determine the length of time immunity persisted when mice are immunized with cell-wall materials isolated from B. tularense or with a living Russian vaccine. At the end of 12 weeks it was no longer possible to detect significant immunity in mice given the cell-wall preparation, whereas at the end of 18 weeks mice given the Russian vaccine still were capable of resisting infection with a moderately virulent strain of this organism. It is interesting to note that the animals vaccinated with Russian vaccine earlier in the course of the experiment had been resistant to infection with virulent strains of organisms, but that this capability was lost as time progressed.

6. When dried tubercle bacilli are administered intramuscularly to mice at the same time that a virulent strain of Pasteurella pestis is given by the same route, the plague infection is subject to interference to approximately the same extent as it is with Rickettsia typhi.

7. Listeria monocytogenes was responsible for infection in a veterinarian who was attending an aborting cow. This organism produced multiple skin lesions in the individual.

8. Studies to determine the incidence of animals in which Pasteurella septica is present in the mouth have been initiated. This organism was isolated from 21 of 65 cats and 1 of 5 dogs. Since about 50 human cases of pasteurellosis due to animal bite have been reported, these studies call attention to cats and dogs as a source of human illness.

9. Histoplasma capsulatum was isolated from a bat (Myotis sp.). This is the first isolation of histoplasma from this group of animals.

Significance:

The number of isolations of rabies made from bats in Montana this year indicates that the problem is greater than was previously supposed. In addition to the fact that a relatively large number of isolations were made, it is important to note that at least 4 species representing 3 genera of bats have been found to be infected with rabies virus. Likewise, the demonstration that infective virus was present in the saliva and that virus could be transmitted by bite indicates that bats are capable of producing disease in hosts that are bitten.

Studies performed on the ability of various vaccines to immunize mice against infections with B. tularense suggest that living organisms are capable of producing a longer lasting and more efficient immunity than can be produced with dead products. While the Russian vaccine is an example of an attenuated strain that might be useful for this purpose, there are many other American strains that might be equally as valuable.

It has been impressive to note the degree of damage which spores of Emmonsia can produce in large laboratory animals and to detect the degree of hypersensitivity and the level of circulating antibodies certain species such as dogs can develop against infections produced by inhalation or injection of viable spores. Previous observations have indicated that this organism was not to be considered a pathogen, but the demonstrations made in this laboratory indicate that it is capable of producing a severe and often fatal disease.

Proposed course:

It is proposed to continue our studies of rabies in bats with the idea of further determining the incidence of the disease in these animals and the methods whereby it is spread from individual to individual. Attempts will be made to institute and to maintain colonies of bats in the laboratory in order to discover more facts about pathogenesis and immunity of this host-virus complex.

Studies will be continued on the various antigenic materials which may be useful in producing immunity against infections with B. tularense. It is hoped that some knowledge may be obtained of the factors which make a living organism a better antigen than those presently available. Studies directed toward immunization against rabies will be continued and attempts will be made to increase the amount of virus available as antigen and to purify preparations for immunization purposes.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Jellison, William L., Stoenner, Herbert G., and Berg, George M.: Leptospirosis among Indians in the Dakotas. Rocky Mtn. Med. Jour. 55(12): 56-58. Dec. 1958.

Jellison, W. L., Epler, Deane C., Kuhns, Edith, and Kohls, Glen M.: Tularemia in man from a domestic rural water supply. Arquivos V Congresso Internacional de Microbiologia, Rio de Janeiro, Aug. 17-24, 1950. 1: 301-304. 1958.

Jellison, William L., Bell, J. Frederick, Vertrees, J. D., Holmes, M. A., Larson, Carl L., and Owen, Cora R.: Preliminary observations on diseases in the 1957-58 outbreak of Microtus in western United States. Trans. 23rd N. A. Wildlife Conf., pp. 137-145. Mar. 1958.

Bell, J. Frederick, Jellison, William L., Owen, Cora R., and Larson, Carl L.: Applicability of the Ascoli test to epizootic tularemia in wild rodents. J. Wildlife Mgmt. 23(2): 238-240. Apr. 1959.

Bell, J. Frederick: Transmission of rabies to laboratory animals by bite of a naturally infected insectivorous bat. Science 129 (3361): 1490-1491. May 1959.

Bell, J. Frederick and Meis, Armon: Pericarditis in infection due to Coxsackie virus group B, type 3. New Eng. J. Med. 261(3): 126-128. July 1959.

Bell, J. Frederick and Hadlow, W. J.: Responses of newborn and adult domestic rabbits (Oryctolagus) to infections with Coxsackie viruses. J. Inf. Dis. 105(1): 54-60. July-Aug. 1959.

Jellison, W. L., Bell, J. F., and Owen, C. R.: Mouse disease studies. In The Oregon Meadow Mouse Irruption of 1957-1958, Federal Cooperative Extension Service, Oregon State College, Corvallis, pp. 71-80. 1959.

In press:

Burgdorfer, Willy: The possible role of ticks as vectors of Leptospirae. II. Infection of the ixodid ticks, Dermacentor andersoni and Amblyomma maculatum, with Leptospira pomona. Exp. Parasit.

Jellison, William L.: Sodoku. Rat-bite fever due to Spirillum minus Carter. Diagnostic Procedures and Reagents, edited by Dr. William B. Cherry, 4th Edition.

Larson, Carl L.: Tularemia. Diagnostic Procedures and Reagents, edited by Dr. William B. Cherry, 4th Edition.

Bell, J. Frederick and Moore, G. John: Rabies virus isolated from brown fat of naturally infected bats. Proc. Soc. Exp. Biol. & Med.

Owen, Cora R., Meis, Armon, Jackson, J. W., and Stoenner, H. G.: A case of primary cutaneous listeriosis. New Eng. J. Med.

Honors and Awards relating to this project:

Dr. H. G. Stoenner

Appointed to editorial board of the American Veterinary Medical Association.

Serial No. NIAID-174
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Transmission of disease agents by certain vectors

Principal Investigator: C. B. Philip

Other Investigators: J. F. Bell, J. M. Brennan, W. Burgdorfer, W. L. Jellison, G. M. Kohls, and D. B. Lackman

Cooperating Units:

Man Years (calendar year 1959):

Total:	11.5
Professional:	3.5
Other:	8

Project Description:

Objectives:

This group directs its studies toward the intimate relationships between infectious agents and their insect vectors. It is desired to obtain an intimate knowledge of the biology, taxonomy, and natural history of insects and other arthropods that are real or potential vectors of pathogenic agents. Efforts during the last year have been directed primarily toward elucidation of tick paralysis and studies of transovarial transmission of Bacterium tularense, Rickettsia rickettsii, and CTF virus by ticks.

Methods:

The methods employed are in most instances those developed at the Rocky Mountain Laboratory. In addition, fluorescent microscopy is being applied to tick tissues in order to follow the course of infection in ticks.

Major findings:

1. It had been previously assumed that there was a single species of tick (Argus reflexus) which infested birds. Recent studies have indicated that this so-called species consists of at least three--one which occurs in Europe, another in Egypt, and a

Part B included Yes X No

third in North America. Since it has been postulated that birds and insects associated with them may be responsible for spread of arbor viruses throughout the world, it is of interest to study the natural history of these species.

2. Cooperative studies with Dr. Esplin, of the University of Utah Medical School, have shown that the early incoordination and paralysis of an ascending nature may be related to the impairment of stretch reflex pathways in hosts afflicted with tick paralysis. This is the first time that it has been shown that central nervous system involvement is present in this disease.

3. Attempts to produce a strain of ticks uniformly capable of transmitting tick paralysis have proved disappointing. If a genetic factor is important, the fact that it is impossible to select male ticks on the basis of ability to transmit disease is probably the underlying difficulty.

4. Adult tropical mites have been infected with the rickettsia of North Queensland tick typhus. Such mites retain infection for at least 49 days. The titer of the rickettsia in the mites at the end of the 42nd day was $10^{5.5}$.

5. There has been considerable discussion concerning the ability of Bacterium tularense to be transovarially transmitted by the tick, Dermacentor andersoni. Studies employing fluorescent microscopy show that B. tularense is present in the germinal ridge of infected female ticks, but organisms cannot be demonstrated in individual egg folicles.

6. R. rickettsii, the cause of spotted fever, can be localized in the tissues of wood ticks.

Significance:

Studies of ticks infected with various pathogenic organisms indicate that it is possible by employing fluorescent microscopy to identify the organism involved and to study the methods and the sites at which these organisms persist. Since in many instances it is difficult or impossible to perform titrations which give satisfactory results regarding the various organs in which microorganisms may persist, the use of fluorescent microscopy appears to be a valuable procedure to aid in determining, for instance, whether or not transovarial transmission of certain agents can occur.

The range of experimental animals that can develop tick paralysis has been extended considerably during the past year. Paralysis has been produced satisfactorily and consistently in

monkeys, woodchucks, dogs, and hamsters. All of these animals develop characteristic symptoms, and the pharmacology and physiology of the disease have been studied in dogs, hamsters, and woodchucks. It is apparent that the pathogenesis of this disease is somewhat different than was previously supposed.

Proposed course:

Studies on the pathogenesis of infectious agents in ticks will be continued with special emphasis being placed on studies of interference between Colorado tick fever virus and R. rickettsii. Investigations of tick paralysis will be continued, special effort being made to determine the relation of the toxin associated with this disease and the occurrence of orotoxin in gravid females. In addition, other species of ticks will be examined to determine whether or not they are capable of producing and transmitting this disease.

At the present time, plans are being laid to carry out a cooperative project between members of this laboratory and the Middle America Research Unit in Panama. This project will be concerned with the ability of mites to maintain and transmit infectious diseases in nature. During the past few years we have been engaged in a study of the biology and taxonomy of mites and this has progressed to a point where we can now intelligently examine this group for their potential ability as important vectors of human and animal disease.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Hughes, Lyndahl E. and Philip, Cornelius B.: Experimental tick paralysis in laboratory animals and native Montana rodents. Proc. Soc. Exp. Biol. & Med. 99(2): 316-319. Nov. 1958.

Philip, Cornelius B. and Aitken, Thomas H. G.: Records of Tabanidae from Sardinia and Corsica. Memorie della Societa Entomologica Italiana 37: 87-97. Nov. 1958.

Philip, Cornelius B.: New records of Tabanidae in the Antilles. Supplemental report. Am. Mus. Novitates No. 1921: 1-7. Dec. 1958.

Brennan, James M.: Synonymy of Odontacarus Ewing, 1929, and Acomatacarus Ewing, 1942, with redescriptions of O. dentatus (Ewing) and O. australis (Ewing), also descriptions of three new species from southern United States (Acarina: Trombiculidae). Ann. Ent. Soc. Amer. 52(1): 1-6. Jan. 1959.

Brennan, James M.: A Croesus among insects. Nat. History 68(1): 56. Jan. 1959.

Brennan, James M. and Jones, Eleanor K.: Keys to the chiggers of North America with synonymic notes and descriptions of two new genera (Acarina: Trombiculidae). Ann. Ent. Soc. Amer. 52(1): 7-16. Jan. 1959.

Brennan, James M. and Loomis, Richard B.: A review of the reptile chiggers, genus Fonsecia (Acarina, Trombiculidae), with descriptions of two new American species. J. Parasitol. 45(1): 53-64. Feb. 1959.

Philip, Cornelius B.: Philippine Zoological Expedition 1946-1947. Tabanidae (Diptera). Fieldiana: Zoology 33: 543-625. Apr. 1959.

Jellison, William L.: Fleas and disease. Ann. Rev. Ent. 4: 389-414. 1959.

Hoogstraal, Harry and Kohls, Glen M.: The Haemaphysalis ticks (Acarina: Ixodidae) of birds. I. H. ornithophila n. sp. from Burma and Thailand. J. Parasitol. 45(4): 417-420. Aug. 1959.

Brennan, James M. and Jones, Eleanor K.: Pseudoschöngastia and four new Neotropical species of the genus (Acarina: Trombiculidae). J. Parasitol. 45(4): 421-429. Aug. 1959.

Philip, Cornelius B.: New North American Tabanidae. X. Notes on synonymy, and description of a new species of Chrysops. Trans. Am. Ent. Soc. 85: 193-217. Oct. 1959.

Philip, Cornelius B.: Some records of Tabanidae (Dipt.) from Iran. Mitteilungen der Schweizerischen Entomologischen Gesellschaft XXXII (2 & 3): 333-336. Oct. 1959.

In press:

Philip, Cornelius B.: The influence of "Wallacea" on the fauna of Tabanidae (Diptera) of the Philippine Archipelago. Pacific Insects.

Philip, Cornelius B.: Malaysian Parasites. XXXV. Description of some Tabanidae (Diptera) from the Far East. Malaysian Parasites.

Philip, Cornelius B.: Malaysian Parasites. XXXVI. A summary review and records of Tabanidae from Malaya, Borneo, and Thailand. Malaysian Parasites.

Philip, Cornelius B.: Canine rickettsiosis in western United States and comparison with a similar disease in the Old World. Arch. Pasteur Inst., Tunis.

Philip, C. B. and Mackerras, I. M.: On Asiatic and related Chrysopinae (Diptera: Tabanidae). Phil. J. Sci.

Philip, Cornelius B.: Further records of Neotropical Tabanidae (Diptera) mostly from Peru. Proc. Calif. Acad. Sci.

Philip, Cornelius B.: Rickettsiae, Microtatobiotes, and Tick Paralysis. Encyclopedia of Science and Technology, McGraw-Hill Book Co.

Brennan, James M. and White, Jesse S.: New records and descriptions of chiggers (Acarina: Trombiculidae) on bats in Alabama. J. Parasitol.

Brennan, James M.: Ectonyx, a new neotropical genus of chiggers (Acarina: Trombiculidae). Acarologia.

Brennan, James M. and Delmat, Herbert C.: Chiggers of Guatemala (Acarina: Trombiculidae). Ann. Ent. Soc. Amer.

Kohls, Glen M.: Ixodidae. Encyclopedia of Science and Technology, McGraw-Hill Book Co.

Kohls, Glen M.: Records and new synonymy of New World Haemaphysalis ticks, with descriptions of the nymph and larva of H. juxtakochi Cooley. J. Parasitol.

Honors and Awards relating to this project:

Dr. J. F. Bell

Appointed counselor for 2 years to Wildlife Disease Association.

Dr. J. M. Brennan

Appointed consultant on American Institute of Biological Sciences Secondary School Biological Science Film Series.

Dr. W. L. Jellison

Invited to edit chapter on Q fever for Merck Veterinary Manual.

Reappointed as Honorary Research Associate of the Smithsonian Institute for a period of 3 years, effective September 14, 1959.

Reappointed to editorial board of the Journal of Wildlife.

Member of editorial board of Wildlife Diseases.

G. M. Kohls

Reappointed to editorial board of the Journal of Parasitology for a 4-year term.

Elected vice-president International Northwest Conference on Diseases in Nature Communicable to Man.

Dr. C. B. Philip

Appointed as one of 14 members of Food and Agricultural Organization of the United Nations Expert Panel on Tick-borne Diseases of Livestock.

Elected to Governing Board, Entomological Society of America, as official delegate from Pacific Coast Branch.

Elected as U. S. representative on the International Committee on Bacteriological Nomenclature.

Appointed to the Price Interagency Survey Committee on BW testing at Dugway Proving Ground.

Serial No. NIAID-175
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Allergy and immunology of fungal infections and the mechanisms of allergic phenomena

Principal Investigator: S. B. Salvin

Other Investigator: R. F. Smith

Cooperating Units:

Man Years (calendar year 1959):
Total: 13
Professional: 4
Other: 9

Project Description:

Objectives:

(1) To study the basic antigen-antibody mechanism responsible for delayed hypersensitivity, (2) to study the relation between delayed hypersensitivity and the formation of circulating antibody, and (3) to determine the effect of delayed hypersensitivity on susceptibility to chronic infectious disease.

Methods:

The methods employed are the classical immunological procedures, both biological and chemical in nature. In addition to the use of such purified materials as egg albumin and diphtheria toxoid, studies are being pursued employing various types of conjugated antigens. The chronic infectious processes under consideration include histoplasmosis, infections due to Candida albicans, Trichophyton mentagrophytes, and T. rubrum.

Major findings:

Previous studies have shown that following the injection of antigen, animals develop delayed hypersensitivity followed by production of circulating antibodies and appearance of immediate hypersensitivity. By administration of total body irradiation

Part B included Yes X No

12-24 hours prior to sensitization it was possible to delay or eliminate production of circulating antibodies, although there was no change in the development of delayed hypersensitivity. It was also shown that if irradiation were delayed for 24 hours after administration of antigenic material, it had no effect upon either delayed or immediate hypersensitivity. These studies led to the idea that delayed hypersensitivity is an essential step in antibody formation. These studies have been continued during the present year.

1. By employing the anamnestic response, further evidence was obtained on the relation of delayed hypersensitivity to antibody formation. A sufficiently small dose of antigen is given to guinea pigs to produce delayed hypersensitivity only. Such animals do not subsequently develop circulating antibodies unless a second injection of antigen is given whereupon an accelerated response occurs. The induction period for antibody development is shortest when the secondary stimulus is administered at the time of maximum delayed hypersensitivity. If the secondary injection is given either before or after this time, the accelerated response is relatively delayed. Total body irradiation 18 hours before administration of either the primary or secondary injection of antigen does not inhibit the anamnestic response, probably because this reponse is dependent upon delayed hypersensitivity which has been shown to be radioresistant.

2. By employing artificial protein conjugates, it has been shown that delayed hypersensitivity is directed toward a different part of the antigen molecule than is immediate hypersensitivity. Animals sensitized to picrylated hen ovalbumin develop delayed hypersensitivity to homologous hapten-homologous protein and heterologous hapten-homologous protein antigens, but do not develop delayed hypersensitivity to homologous hapten-heterologous protein conjugates. When these animals develop circulating antibodies, however, they become sensitive to conjugates containing homologous haptens even though heterologous proteins are contained in the conjugate. By employing the anamnestic response procedure again, it can be shown that maximal anamnestic response occurs when homologous hapten homologous-protein is used as a secondary stimulus and that heterologous hapten-homologous protein conjugates produce about the same degree of anamnestic response. When, however, homologous hapten-heterologous protein is employed, the anamnestic response is minimal.

3. While others have shown that the antimetabolite 6-mercaptopurine inhibits the development of circulating antibodies in rabbits, we have been unable to substantiate these results employing guinea pigs as test animals. Administration of this compound in quantities up to 75 mgms per kilo per day does not prevent the appearance of either delayed or immediate hypersensitivity to diphtheria toxoid.

4. A highly purified antigen obtained from Trichophyton rubrum is being used as a skin test agent in patients having dermatophytosis due to Trichophyton mentagrophytes and T. rubrum. Patients with infections due to T. mentagrophytes display delayed hypersensitivity to the purified antigen, whereas patients with infections due to T. rubrum do not manifest delayed hypersensitivity. It may be possible that T. rubrum causes chronic type lesions because it lacks an antigen that is capable of producing hypersensitivity and also immunity.

Significance:

The data made available indicate that delayed hypersensitivity is an early and essential phase in the development of classical immunity. We have found that delayed reactions occur prior to the appearance of circulating antibodies. We also have found that those antigens effective in producing delayed hypersensitivity are capable of producing antibody, whereas antigens ineffective in producing delayed reactions produce little antibody. The fact that the anamnestic reaction is more pronounced when the secondary stimulus is given at the peak of delayed hypersensitivity likewise supports the idea of the close relationship of delayed and immediate hypersensitivity. With conjugated antigens it can be demonstrated that delayed hypersensitivity is related to the protein moiety of the antigen, whereas with immediate hypersensitivity the reaction is related to the hapten portion of the conjugate. If proposed experiments yield evidence consistent with past experience, it will be necessary to alter the present classical concept of the specificity of antigen-antibody reactions, since it will have been shown that specificity changes as the antibody reaction matures.

Proposed course:

Experiments will be conducted to determine those chemical groupings that can be differentiated by delayed hypersensitivity and those that can be differentiated by circulating antibody or immediate hypersensitivity.

Attempts will be made to detect delayed hypersensitivity to purified polysaccharides. Although it is known that circulating antibodies may be demonstrated against polysaccharides, delayed hypersensitivity has not been elicited. There is reason to believe that polysaccharides conjugate with host protein and therefore in order to elicit delayed reactions one would have to employ the protein itself. Experiments will be carried out with artificially conjugated protein-polysaccharides to test this hypothesis.

Studies are being performed, employing histochemical methods, to determine the cells and the cell systems involved in the various phases of the antibody process.

Dogs have been immunized with various antigenic fractions of Histoplasma capsulatum and infected with spores by the pulmonary route. Immunogenesis and pathogenesis of histoplasmosis are being studied in these animals.

Studies will be continued on the role of delayed and immediate hypersensitivity in immunity to dermatophytosis. Subjects who have been desensitized with purified antigens or hypersensitized with an antigen-antibody complex in antibody excess will be examined.

By the use of highly purified poliovirus as antigen, an attempt will be made to determine if delayed hypersensitivity can be developed in guinea pigs and, if it is present, to determine if hypersensitivity to one type may be the basis for increased immunity to the other two types.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Salvin, S. B.: The influence of leucocytes from sensitized mice on resistance to Histoplasma capsulatum. Am. J. Hyg. 68(3): 233-241. Nov. 1958.

Salvin, Samuel B.: Delayed-type hypersensitivity and antibody formation--a discussion. In Mechanisms of Hypersensitivity, Little, Brown & Co., Boston, Mass. pp. 450-452. 1959.

Salvin, S. B. and Smith, Robert F.: Delayed hypersensitivity in the development of circulating antibody. The effect of X-irradiation. J. Exp. Med. 109(4): 325-338. Apr. 1959.

Salvin, S. B.: Current concepts of diagnostic serology and skin hypersensitivity in the mycoses. Am. J. Med. 27(1): 97-114. July 1959.

Salvin, S. B. and Smith, R. F.: Antigens from the yeast phase of Histoplasma capsulatum. III. Isolation, properties, and activity of a protein-carbohydrate complex. J. Inf. Dis. 105(1): 45-53. July-Aug. 1959.

In press:

Salvin, S. B. and Smith, R. F.: Delayed hypersensitivity and the anamnestic response. J. Immunol.

Salvin, S. B.: Resistance of animals and man to histoplasmosis. Histoplasmosis, edited by Dr. Henry C. Sweany, Chas. C. Thomas, Springfield, Ill.

Serial No. NIAID-176
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Viruses, virus components, and virus surfaces

Principal Investigator: B. H. Hoyer

Other Investigators: R. K. Gerloff, F. G. Jarvis, R. A. Ormsbee, and D. B. Ritter

Cooperating Units:

Man Years (calendar year 1959):
Total: 11.5
Professional: 3.5
Other: 8

Project Description:

Objectives:

Investigations are directed toward determining how animal viruses proliferate in tissue culture cells. The sites of production of nucleic acid and protein and the systems which function to produce these viral constituents are subjects of study. Ancillary to these broad objectives are studies of methods of labelling various materials used as "building blocks" in newly-formed virus and of methods of purifying virus preparations easily and rapidly without undue loss.

Methods:

These studies are performed using quantitative and qualitative methods applicable to studies of cell metabolism and virus growth. Chromatographic separation of cell constitutents, viruses, and viral products has been developed and utilized. Radioactive labelling of the nucleic acid and protein fractions has been accomplished by incorporating P^{32} and S^{35} into the respective portions of the virus moiety.

Major findings:

1. The main requirements for adsorption of poliovirus to cellulose ion exchange systems are (a) lack of phosphate in the

Part B included Yes X No

suspending buffer or in the column and (b) use of an organic buffer such as TRIS as the suspending medium. Type 3 virus may then be eluted by 0.05M added NaCl and types 1 and 2 by 0.10M added NaCl. Type 3 virus may be eluted at pH 7.1 and types 1 and 2 at pH 6.6. Addition of formaldehyde changes the elution properties of type 2 but not of types 1 and 3 polioviruses.

2. By applying some of the above findings, it has been possible to obtain large yields of pure poliovirus. As much as 4 liters of fluid can be concentrated to 300 ml by adsorption of dialyzed tissue culture fluids to DEAE columns equilibrating with 0.01M TRIS-HCl at pH 8.2 and eluting with 0.15M NaCl. Crystalline virus may then be harvested by methods previously described. A routine harvest of 5 X 10" PFU of virus is obtained.

3. Radioactive methionine (S^{35}) has been prepared from Torula utilis grown in synthetic medium. The amino acid is purified by chromatographic methods. The uptake of this product by tissue culture cells is in the neighborhood of 60 to 80%.

4. The protein of type 2 poliovirus has been labelled with S^{35} which is incorporated as radioactive methionine added to tissue culture medium. All S^{35} remains in methionine and about 1 count per minute per 10^4 PFU is found. Upon chromatography of labelled virus it is found that PFU, S^{35}, and P^{32} peaks coincide. 3% of the S^{35} incorporated into the nondialyzable portions of the tissue culture cell appears in the final virus particle.

5. Dissociation of whole poliovirus particles may be accomplished simply by freeze-drying. Centrifugal analysis of virus shows a homogeneous peak sedimenting at 160S. After lyophilization this peak disappears, and a diffusely sedimenting material and a fraction sedimenting at 70S are noted.

6. A recovery of 95% of RNA contained in labelled purified virus has been obtained after extraction with phenol. Only 1% to 2% of the RNA, when placed on tissue culture cells becomes bound and only 0.01% of bound RNA is infective.

7. Radio-suicide experiments indicate that the reason RNA is relatively inactive is due to destruction of the integrity of the RNA molecule by virtue of the incorporated P^{32}.

8. By employing concentrated labelled poliovirus and hyperimmune monkey serums in precipitin tests, it was possible to show that high-titered serum caused precipitation of virus in a specific manner.

Methods of purification arrived at make it possible to produce crystallized poliovirus from large batches of infective fluid (up to 4 liters have been processed). Based upon 10^6 PFU/ml of virus as a satisfactory amount for immunization purposes and upon routine production of 5 X 10" PFU of virus, it is evident that 500,000 doses of virus suitable for inactivation for use as vaccine can be made even with the limited resources available.

Poliovirus may now be labelled specifically with P^{32} and S^{35} so that the role of nucleic acids and proteins in the life cycle of this virus can be investigated. Since 3% of the S^{35} incorporated into the large molecules of tissue culture cells appears in new virus, the method of labelling virus protein suggests that it should be possible to differentiate virus and cell proteins by studying their specific activity (units S^{35}/units protein).

The studies of radio-suicide of poliovirus suggest that only 1 band need be broken in the RNA molecule in order to inactivate it and thus destroy activity. Results indicate that there is only one RNA per virus particle and that this is in the nature of a single strand molecule. This is in contrast to the DNA molecule of bacteriophage which is in the nature of a spiral molecule and requires the disruption of 10 bands before it is inactivated.

Proposed course:

Since the surface qualities of the types of poliovirus can be distinguished, it is planned to study those of the "avirulent" strains, being developed for vaccine purposes by others, to determine if they differ in their chromatographic characteristics. This might conceivably be of aid in field investigations concerning the safety of such vaccines.

Attempts will be continued to study the various phases of the life cycle of viruses with specific attempts to determine the sites at which virus protein synthesis occurs and the methods whereby this is accomplished. The discovery that when the specific activity of P^{32} approaches 200 to 300 millicuries/mg P, tissue cells cease to produce virus, even though such amounts of radioactivity fail to influence whole virus particles, suggests that RNA is extremely sensitive to radioactivity. Based on these observations and upon the fact that P^{31} competes with P^{32} and interferes with incorporation of radioactive material in RNA the opportunity to investigate the time sequence of RNA synthesis is afforded.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Hoyer, Bill H., Bolton, Ellis T., Ritter, Daniel B., and Ribi, Edgar: Simple method for preparation of purified, radioactive poliovirus particles: electron micrograph. Virology (Notes) 7(4): 462-464. Apr. 1959.

Serial No. NIAID-177
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Immune prophylaxis of mycobacterial infections

Principal Investigator: E. Ribi

Other Investigators: W. T. Haskins and C. L. Larson

Cooperating Units:

Man Years (calendar year 1959):
Total: 4.75
Professional: .75
Other: 4

Project Description:

Objectives:

The principal purpose of this study is to obtain information regarding immunity against infections with Mycobacterium tuberculosis in experimental animals and to determine and isolate those entities which produce hypersensitivity and protective immunity. Additionally, the need for a test which will adequately test the potency of live vaccines is being considered and investigated. Emphasis has been placed upon the role of cell walls and protoplasm of acid-fast organisms in inducing and provoking delayed hypersensitivity and in their ability to potentiate immunogenic response when incorporated into Freund's adjuvant.

Methods:

A system for mechanical disruption of bacteria has been developed and this is employed for primary separation of protoplasm and cell walls of various acid-fast organisms. Production of iso-allergic encephalitis in guinea pigs is used to test the efficacy of fractions incorporated into Freund's adjuvant to produce immunity. A variety of challenge methods including aerosol infections with large and small numbers of virulent organisms, intravenous injection, and intracorneal inoculation are used to study the immunogenicity of various materials in mice. Rabbit skin tests are employed in studies concerning delayed hypersensitivity.

Part B included Yes X No

1. Cell walls from Mycobacterium sp. can be readily purified and the yield doubled by sedimentation in a continuous linear sucrose density gradient.

2. Iso-allergic encephalitis can be produced in guinea pigs immunized with brain antigen contained in Freund's adjuvant in which cell walls are incorporated. The effect of protoplasm is not so evident. Thus, when antigen is administered subcutaneously into the nape of the neck, 0.1 mg of cell walls is sufficient to produce an effect, while 1.0 to 2.0 mg of protoplasm is ineffective. When administered in the foot pad, 0.005 mg of cell walls is as effective as 0.1 mg of protoplasm.

3. Rabbits sensitized by intradermal injection of 10 to 100 µg of cell walls of either M. butyricum or M. tuberculosis develop specific sensitivity to the organism administered. Animals given M. butyricum develop lesions against doses of cell walls of the homologous organism which are 100- to 500-fold smaller than amounts of cell walls of the heterologous organism required to produce lesions. In the case of animals sensitized to M. tuberculosis, the dose of heterologous cell walls required to produce a delayed reaction is 4 to 8 times larger than the dose of homologous cell walls.

4. Cell walls, protoplasm, and phenol-extracted cells fail to produce evidence of satisfactory immunity in mice challenged with an aerosol containing 10 to 100 infectious doses if virulent tubercle bacilli.

5. Cell walls, protoplasm, and phenol-extracted cells produce resistance in mice subsequently challenged with an aerosol containing 1,000 to 10,000 infectious doses of virulent bacilli or inoculated by the intracorneal or intravenous route.

Significance:

Present experiments, in which mice were used as experimental animals, give divergent results regarding the ability of dead or live vaccines to confer immunity. Mice challenged with a small number of virulent bacilli by aerosol and later examined for the presence of pulmonary lesions and of virulent organisms in the lungs and spleen are found to be resistant only if vaccinated with living avirulent organisms. When immunized with dead cells or fractions thereof, mice show no evidence of immunity when challenged by this method. If, on the other hand, immunized mice are challenged by intracorneal inoculation or with massive doses of organisms given intravenously or by aerosol, and death is taken as the criterion of susceptibility, then it is possible to show that a variety of killed products are capable of producing

resistance. The results obtained by the first experimental method more nearly duplicate results obtained following vaccination of human populations with live vaccines and this suggests that this is a more rational method for testing immunogenic materials against tuberculosis.

Methods are now available for producing large quantities of cell walls and protoplasm from a variety of acid-fast bacilli. The effect of these materials as sensitizing and immunizing agents can now be tested more thoroughly as they are available in suitable amounts. Experiments showing that cell walls are the active portion of the mycobacteria in complete Freund's adjuvant were performed after the new techniques were developed.

Demonstration of the specificity of M. butyricum and M. tuberculosis in producing and eliciting sensitivity suggests that it may be possible to differentiate various species of mycobacteria by this method and to develop methods which might serve to aid in diagnosis of infections due to members of this group.

Proposed course:

Further attempts will be made to immunize a variety of animals against tuberculosis infection by employing killed bacilli and fractions isolated from mycobacteria. There is some evidence that use of solubilized whole material may be effective. It is likewise possible that the choice of experimental animals may considerably influence the outcome of attempts at immunization. It has been suggested that since rabbits have a certain degree of native resistance to infection, they may be the animal of choice for these studies. Further attempts will be made to isolate and identify the sensitizing portion of the tubercle bacillus in order that it may be eliminated from the fraction to be used as an immunogenic agent.

Studies of the specificity of hypersensitive states produced by various species of mycobacteria will be extended to include more species and will be augmented by serological examination performed with specific serums and with antigens extracted from various organisms.

The role of cell walls as portions of complete antigen will be further examined, especially to determine if the factor in cell walls which contributes to potentiation of immunity is identical or related to that which produces hypersensitivity.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Ribi, Edgar, Perrine, Theodore, List, Robert, Brown, William, and Goode, Granville: Use of pressure cell to prepare cell walls from mycobacteria. Proc. Soc. Exp. Biol. & Med. 100(3): 647-649. Mar. 1959.

In press:

Ribi, Edgar, Brown, William, and Good, Granville: Preparation of microorganisms for electron microscopy. J. Bact.

Serial No. NIAID-178
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Immune prophylaxis of Salmonella infections

Principal Investigator: E. Ribi

Other Investigators: W. T. Haskins, F. G. Jarvis, and K. C. Milner

Cooperating Units:

Man Years (calendar year 1959):
Total: 5.5
Professional: 2.5
Other: 3

Project Description:

Objectives:

Immunity against many infections produced by Gram-negative organisms is intimately related to the endotoxins they produce, and in the case of typhoid fever, the role of Vi antigens also must be considered. Studies are directed toward an intimate knowledge of the chemical structure of endotoxins or O antigens, development of methods of producing and purifying Vi antigen in its native state, understanding of the role of specific serologic factors of O antigens in production of protective immunity, and development of laboratory tests which will make it possible to analyze the part played by various antigens in inducing protection against infections with Gram-negative bacteria.

Methods:

Biophysical, biochemical, and biological approaches are combined in this project. Physical and mild chemical treatments are applied to bacteria to isolate various antigenic fractions, and such techniques as curtain electrophoresis are employed to purify products. Electron microscopy is performed as a routine "control" procedure.

Major findings:

1. Delipified toxins (those in which the lipid content has been reduced from approximately 19% to 1% or 2%) are equally

Part B included Yes X No

as toxic and pyrogenic as natural endotoxins. In addition, they are equally as lethal for thorotrast-treated rabbits and as active in producing tumor damage in mice. Both preparations induce specific protection in mice.

2. Lipid freed from endotoxin by acid hydrolysis stimulates nonspecific resistance. Since this material contains up to 20% carbohydrate and a small amount of amino acids, it is possible that certain sugars or a combination of sugars with amino acids may contribute to nonspecific resistance.

3. Endotoxins may be delipified and deproteinized to an irreducible minimum of approximately 1% lipid and 1% protein without loss of toxicity. These delipified toxins are capable of stimulating nonspecific as well as specific resistance. The lipid extracted does not produce specific resistance.

4. A survey of various organic liquids as solvents for the endotoxin of Salmonella enteritidis indicates that a number of solvents extract endotoxins without affecting their potency. Endotoxin is remarkably stable to reagents which are known to react with hydroxyl and amino groups but is partially or wholly destroyed by formic acid and lactic acid. Liquefied phenol was found to be an excellent solvent as was dimethyl sulfoxide.

5. In studying the specificity of active protection stimulated by O antigens, it has been found that while a standard whole cell preparation and an antigenically complete toxic extract are capable of protecting mice against infection with S. enteritidis, haptenes, lipids, and unrelated endotoxins were incapable of conferring protection.

6. The particular serological factors that are essential to stimulate protection against S. enteritidis have been determined. Only organisms containing factor 9 and a major portion of factor 12 will produce immunity when administered to mice.

7. It has been shown by the use of radiosulfur that Vi antigen contains no sulfur atoms. Vi antigen labelled with radioactive carbon may be separated from other antigenic constituents of organisms and purified by curtain electrophoresis. Results of viscosity and sedimentation-rate studies demonstrate that the Vi antigen molecule under investigation is larger and less polydisperse than that isolated by Webster et al. Studies of the protection conferred by Vi antigen indicate that it acts as an anticomplementary substance and, consequently, may promote infection by otherwise nonvirulent organisms.

According to the German school, the biological activity of endotoxins is attributable primarily to the lipid component of the carbohydrate-lipid-protein complex. Results of studies performed at the Rocky Mountain Laboratory show that the specific activity of endotoxins is due to carbohydrate, for these activities are possessed by endotoxins from which lipid and protein have been largely extracted leaving only about 1% of these components combined with carbohydrate. Since protective immunity can be induced by administration of such materials to mice, it is reasonable to assume that by experimental procedures the specific portion of the carbohydrate responsible for activity of endotoxins may be isolated, identified, and practically applied.

The use of the S. enteritidis system for studying protective immunity has made it possible to detect the serologic factors of importance. As an example, S. enteritidis contains O factors 1, 9, 12_1, 12_2, and 12_3; S. typhosa has factors 9 and complete 12 complex but lacks factor 1; S. bulawayo contains only factor 1 while S. gallinarum contains factors 9, 12, and 12_3. S. enteritidis and S. typhosa are capable of protecting mice, and a graded dose response may be demonstrated. S. gallinarum protects mice to a lesser extent, but S. bulawayo fails to confer protection.

Isolation and purification of Vi antigen by the methods employed here show that previously reported products are degraded to some extent since the present preparations are less polydispersed. These antigens apparently act as anticomplementary substances and serve to increase pathogenicity of S. typhosa for mice in the same way as does gastric mucin. It is felt the essential factor in producing immunity is the endotoxin moiety, but it is possible that Vi antigen may have some effect in man not demonstrable in experimental systems.

Proposed course:

The results of the last few years' work on immunity against Salmonella infections in experimental systems has increasingly strengthened the thesis that endotoxin is the factor responsible for the production of specific protective immunity. Because of these results, analysis of fractions of endotoxin for their ability to act in this capacity will be continued and expanded with a view toward isolating and identifying the active portion of the molecule and of coupling this material to a carrier to constitute an "artificial antigen." The implications of such research, if productive of positive results, can easily be appreciated.

In view of results obtained with experimental systems, it is proposed to arrange for injection of relatively nontoxic material, such as that obtained by treatment of organisms with dioxane, into human subjects to determine toxicity and antigenicity of the preparations. These studies are essential before these antigens could be applied in the field where they may be tested for their efficacy in preventing Salmonella infections in man.

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Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Ribi, Edgar, Milner, Kelsey C., and Perrine, Theodore D.: Endotoxic and antigenic fractions from the cell wall of Salmonella enteritidis. Methods for separation and some biological activities. J. Immunol. 82(1): 75-84. Jan. 1959.

Casey, Mary L. and Smith, Bettie: A family outbreak of Shigellosis. Rocky Mtn. Med. Jour. 56(7): 33-35 and 46. July 1959.

In press:

Ribi, Edgar, Hoyer, Bill H., Milner, Kelsey C., Perrine, Theodore D., Larson, Carl L., and Goode, Granville: Physical and chemical analysis of endotoxin from Salmonella enteritidis. J. Immunol.

Larson, Carl, Ribi, Edgar, Milner, Kelsey C., and Lieberman, Jacob E.: A method for titrating endotoxic activity in the skin of rabbits. J. Exp. Med.

Serial No. NIAID-179
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigations of the role of morphological elements of microorganisms in immunity and related phenomena

Principal Investigator: E. Ribi

Other Investigators: C. L. Larson and K. C. Milner

Cooperating Units:

Man Years (calendar year 1959):
Total: 3.5
Professional: .5
Other: 3

Project Description:

Objectives:

Studies under this section include those done on a variety of organisms as requested by investigators who have become interested in Dr. Ribi's biophysical approaches to microbiology and immunology. Drs. Gerhardt and Lund, of the University of Wisconsin, were desirous of isolating and purifying the morphological elements of spores of Bacillus subtilis and B. terminalis for antigenic and chemical analysis. Dr. Munoz, University of Montana, wished to determine whether cell walls or protoplasm of Bordetella pertussis contained toxin, histamine sensitizing factor, or immunizing antigen. Dr. Foster, of the University of Alabama, worked in close cooperation with members of the staff of the Rocky Mountain Laboratory to determine the morphological site of toxins and protective antigens of Brucella sp.

Methods:

The studies were guided primarily by the techniques developed at the Rocky Mountain Laboratory. These include the present pressure cell system for rupturing intact cells and the use of electron microscopy as a control mechanism. Suitable biological tests were employed as needed.

Part B included Yes X No

1. The toxin of B. pertussis was found to be present in protoplasm. This portion of the cell was toxic both for mice and for the skin of rabbits. The histamine sensitizing factor and the protective antigen were found to be present in the cell walls.

2. The entire spores of B. subtilis and B. terminalis were subjected to mechanical pressure and subsequent differential centrifugation resulting in isolation of spores and of the exosporium coats. Subsequent treatment of these spores by ultrasonic vibration and differential centrifugation allowed for isolation of the spore walls and solid, seemingly wall-less, oblong protoplasmic bodies.

3. Cell wall and protoplasmic fractions were made from B. abortus by the pressure-cell technique. A water-soluble and a bacterial residue fraction were also obtained by extraction of whole cells with ether. These were treated for their ability to protect mice against a virulent B. abortus infection. The protective index was as follows: controls = 0; protoplasm = 1.0; acetone dried whole cells = 1.9; residue of aqueous ether extract = 2.7; cell walls = 3.7; and aqueous ether extract = >4.6. The latter preparation is also a potent endotoxin having an LD_{50} for mice of 0.5 mg.

Significance:

The various fractions prepared from spores will be utilized by scientists in Wisconsin for further immunologic and chemical studies. The separations made so nicely serve to emphasize the wide range of applicability of the present methods and will serve to provide materials useful in studies of the interesting phenomenon of spore formation.

The demonstration that the cell walls of B. pertussis contain the effective protective antigen while the protoplasm contains toxin lethal for mice and capable of producing dermal necrosis in rabbits suggests that vaccines which will confer immunity without producing serious side effects may be developed.

The studies with B. abortus are of interest for a number of reasons. The antigen obtained by extraction with ether is soluble in water and is toxic. Salmonella sp. contain similar antigens but antigens obtained from B. tularense by this treatment are particulate and nontoxic. The demonstration that cell walls and the above extracts are capable of producing immunity in mice serves to emphasize the practicability of employing these as antigens for preventive purposes.

Proposed course:

Since these studies were initiated on a short-term basis as cooperative projects, no plans are made for further specific

investigations of the spore problem or of B. pertussis. It is planned to investigate further the various immunogenic parts of B. abortus to determine whether combining them will produce an interfering, additive, or synergistic effect.

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Munoz, J., Ribi, Edgar, and Larson, Carl L.: Antigens of Bordetella pertussis. I. Activities of cell walls and protoplasm. J. Immunol. 83(5): 496-501. Nov. 1959.

Serial No. NIAID-180
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The encephalitides

Principal Investigator: C. M. Eklund

Other Investigators: W. Burgdorfer, W. L. Jellison, G. M. Kohls, D. B. Lackman, and L. A. Thomas

Cooperating Units:

Man Years (calendar year 1959):
Total: 15.5
Professional: 2.5
Other: 13

Project Description:

Objectives:

The objectives of this program are to determine the incidence of arbor virus infections in the western United States, to develop better methods of diagnosis of these diseases, to develop a detailed knowledge of the ecology and epidemiology of this group of diseases, and to develop general and specific methods of control.

Methods:

The methods employed are in general those used in field studies of arthropod-borne infections and virological and serological techniques suitable for this type of study.

Major findings:

1. Mosquitoes, C. tarsalis, were not abundant in Idaho, Washington, or North Dakota last summer. No isolations of WEE virus and 2 isolations of SLE virus were made from approximately 2,000 C. tarsalis collected in Idaho. Neither of these viruses were isolated from 600 mosquitoes collected in Washington, but a previously unknown virus was obtained from one lot. Four isolations of western virus were made from 2,100 C. tarsalis collected in North Dakota.

Part B included Yes X No ___

2. Attempts to isolate WEE virus from the serums of mammals, birds, and snakes or from various tissues yielded negative results. These results create doubt as to the existence of latent infection in birds under natural conditions.

3. Antibody studies were performed on serums from various birds, animals, and human beings collected during 1958. Serums from turkeys and hogs living in the Bismarck-Mandan area of North Dakota were found to have a high incidence of antibodies against WEE but a very low incidence of antibodies against SLE. It appears that there are focal areas in which WEE virus is active even though the over-all activity in the state is low.

Pigeon serums were collected in Minnesota. The incidence of birds with antibodies against WEE varied from 0 to 70%. Birds in the southern part of the state were found to be essentially negative, whereas about 70% of those in the northern part of the state yielded serums having antibodies.

4. The strain of Powassan virus isolated in Canada was studied in the laboratory. There appears to be but slight relationship between this virus and Russian spring-summer encephalitis and louping ill viruses. The LD_{50} for 21-day-old mice varies from 7.5 to 9.7. It is uniformly infectious no matter which route of inoculation is employed. In 4-day-old mice the LD_{50} varies between 9.5 and 10 when the virus is administered intracerebrally or intraperitoneally. It is highly infectious for 1-day-old chicks causing a viremia persisting for a period of 8 days. Blood from chicks attains a titer of 10^7. Serums from various areas in the West and from Alaska have been examined for the presence of antibodies against Powassan virus with negative results.

5. An attempt is being made to classify arbor viruses by use of criteria which may be helpful in elucidating the natural history of these viruses and, consequently, in studying the ecology and epidemiology of the diseases with which they are associated. The following examples are given: WEE virus produces severe and prolonged viremia in birds, SLE virus produces only a mild viremia, and trivittatus and inornata viruses fail to produce viremia. Of 22 viruses examined for their ability to produce viremia in garter snakes and to produce infection when inoculated intraperitoneally into 21-day-old mice, it was found that 13 failed to react in either host. Two viruses were negative when injected into mice but produced viremia in snakes. There were 7 viruses which produced viremia in snakes and were infective for 21-day-old mice.

6. Studies on the pathogenesis of central nervous system lesions produced in mice by Colorado tick fever virus and SLE virus indicate that the mechanism whereby animals are protected from death

is completely different with each of the viruses. In the case of Colorado tick fever there is a relative insusceptibility of nerve system cells which is acquired with maturation of the animals, whereas with SLE virus the serologic mechanisms which clear the blood and other tissues are likewise responsible for clearing the central nervous system.

7. A complete transmission cycle has been demonstrated between T. sexualis, garter snakes, and WEE virus. Previously we demonstrated that mosquitoes were capable of infecting snakes with the production of a viremia but were unable to demonstrate that mosquitoes feeding upon infected snakes could become infected and thereafter transfer the infection to chicks or other snakes. This phase of the cycle has been completed during the present experiments. It has not been possible to detect antibodies in the blood of snakes which had recovered from viremia produced by injection of WEE virus.

8. The HI test may be used as a satisfactory screening test for the presence of antibodies against SLE and WEE viruses.

Significance:

Negative results in attempts to isolate WEE virus from tissues of birds collected in areas where infection with this virus is endemic throw reasonable doubt upon the importance of latent infections of birds as an important factor in the maintenance of virus in nature. Isolation of virus from the first brood of C. tarsalis and its continuous presence in them as long as appreciable numbers persist further emphasize the role of mosquitoes in this respect.

Simultaneous studies of mosquitoes for the presence of SLE and WEE viruses and surveys for the occurrence of antibodies against these viruses in serums from human beings suggest that while C. tarsalis is the vector of WEE virus there is a distinct possibility that this mosquito is not the important insect vector of SLE virus.

Recent efforts to classify virus on some basis other than the serologic ones suggested by Casals were prompted by the need to have information available which would serve as an aid in ecological investigations. If a virus such as WEE produces viremia in mammals, birds, and snakes, field studies must be directed toward a wide variety of animals in order to determine the natural history. If, on the other hand, such viruses as trivittatus and inoxoata do not infect birds and produce viremia in young mammals only, the area of inquiry may be more restricted.

Studies with Powassan virus indicate that it has at most a remote relation to Russian spring-summer encephalitis virus. The cooperative field study carried out in the area about Powassan, Ontario, indicated that ticks were and have been scarce and that the ecological facts pointed toward some vector other than ticks.

Proposed course:

There is a need for vaccines against certain of the arbor virus infections. Since it has been shown that a 10% suspension of brain from suckling mice infected with CTF virus can be inactivated with 1:4000 formaldehyde at 4° C without loss of antigenicity, it is proposed to extend observations to include other viruses. Preliminary work indicates that louping ill virus may be inactivated in this way. Attempts will be made to select variants of WEE and SLE viruses that have diminished affinity for central nervous system tissue. These also will be subjected to the above inactivation process.

Further studies of the serology of the arbor viruses, together with the pathogenesis in mosquitoes, ticks, and in mammalian and avian hosts, will be continued and expanded. These studies have application to various field problems and also serve to explain many of the varied clinical aspects of the diseases caused by the different viruses.

Attempts are under way to determine whether or not snakes and C. tarsalis, both infected and noninfected, can, under controlled situations existing in nature (root cellars), maintain WEE virus throughout the winter season. Solution of the over-wintering mechanism of virus would be most valuable in providing measures for the control of human disease. Field studies will be continued with especial emphasis upon the periods just before mosquitoes emerge and at the time mosquitoes become infrequent in the late summer or early fall.

Serial No. NIAID-180

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Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Thomas, Leo A., Eklund, Carl M., and Rush, William A.: Susceptibility of garter snakes (Thamnophis spp.) to western equine encephalomyelitis virus. Proc. Soc. Exp. Biol. & Med. 99(3): 698-700. Dec. 1958.

Rush, William A., Brennan, James M., and Eklund, Carl M.: A natural hibernation site of the mosquito, Culex tarsalis Coquillett, in the Columbia River Basin, Washington. Mosq. News 18(4): 288-293. Dec. 1958.

In press:

Thomas, Leo A. and Smith, James V.: The encephalitis outbreak in northern Utah: 2. Infection rates in birds, mammals, and mosquitoes. Mosq. News.

Honors and Awards relating to this project:

Dr. C. M. Eklund

Member of Section Council, Epidemiology Section, American Public Health Association.

Serial No. NIAID-181
Rocky Mtn. Lab.
Hamilton, Mont.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Colorado tick fever

Principal Investigator: C. M. Eklund

Other Investigators: W. Burgdorfer, W. L. Jellison, G. M. Kohls, D. B. Lackman, and L. A. Thomas

Cooperating Units:

Man Years (calendar year 1959):
Total: 18.75
Professional: 2.75
Other: 16

Project Description:

Objectives:

The objectives of this program are to develop a knowledge of the clinical syndromes which CTF virus may reproduce in human beings, to understand the ecology of this disease with special reference to the ticks (Dermacentor andersoni) that transmit the disease and the rodent hosts responsible for maintenance of the disease in nature. In addition, attempts to prevent the disease by means of a prophylactic vaccine are being made.

Methods:

The methods employed do not differ greatly from those used in laboratories studying field and laboratory aspects of arthropod-borne viruses except as they are adapted to our local situations.

Major findings:

1. Up to the present time CTF virus has been isolated from a total of 553 patients. This is a remarkable number of isolations for an arthropod-transmitted virus disease.

2. Surveys for the presence of antibodies against CTF virus have been conducted on serums collected in Wyoming and

Part B included Yes X No ___

Idaho. Approximately 8% of serums from Wyoming and 3% of those from Idaho were found to contain specific antibodies. These findings confirm the impression that subclinical infection is rarely encountered and that disease due to this virus is more common than ordinarily supposed.

3. Studies of the ecology of CTF confirm our previous findings that microecological environments influence the incidence of CTF virus in both native rodents and mature and immature ticks (D. andersoni). Areas in which no virus can be detected either in ticks or in rodents continue to yield negative results, whereas those in which virus has been detected show the continued presence of virus in animals and arthropods although the incidence may fluctuate greatly. It also is interesting to note that the majority of isolations of virus from rodents is from golden-mantled ground squirrels, primarily from immature animals.

4. It has been demonstrated that the Columbian ground squirrel, the golden-mantled ground squirrel, chipmunks, and deer mice may become infected with CTF virus after the bites of infected D. andersoni. All of these animals develop a viremia. It also has been shown that viremia in the chipmunk persists for a period of about 2 weeks, whereas virus can be demonstrated in the blood of the other animals for periods varying from 3 to 6 weeks. The efficiency of the golden-mantled ground squirrel as a mechanism for the maintenance of virus is emphasized by the relatively high titer of virus present in the blood and the length of time viremia persists.

5. Fluorescent antibody technique has been shown to be valuable for identification of CTF virus in suckling mice and for study of the pathogenesis of this disease in experimental animals. A specific fluorescence can be demonstrated in those tissues of experimental animals from which virus can subsequently be isolated.

6. A vaccine which will successfully produce antibodies in experimental animals and volunteer human subjects has been developed. Virus contained in mouse brain is inactivated with 1:4000 formaldehyde at 4° C. Virus is progressively killed, and after a period of 55 to 60 days no virus can be demonstrated in the vaccine either by injection into experimental animals or into volunteers. This material produces satisfactory neutralizing antibodies in humans and protective antibodies in suckling mice and hamsters.

Significance:

As has been emphasized in the past, CTF infection in man is not as mild a disease as has previously been supposed. Frequently encephalitis and bleeding tendencies of greater or lesser severity are encountered. Through the findings of this study the importance

of this disease has been stressed for public health and medical authorities in the West. The demonstration that in the neighborhood of 3 to 8% of individuals residing in rural areas have antibodies against this disease likewise indicates that this is not so rare a condition as we have been lead to believe.

The finding that transovarial transmission does not occur in ticks emphasizes the importance of native rodents as the definitive host of this virus and as the link in the chain of infection toward which control measures should be directed.

It is of great interest that vaccines can be prepared which will produce antibodies in both man and experimental animals. These vaccines are prepared by adding 1:4000 formaldehyde to the virus suspension and allowing them to react at temperatures of 4° C until the virus is killed. This procedure does not impair antigenicity of the virus. Antigenicity is markedly reduced if formaldehyde is allowed to react with virus at temperatures of 27 or 32° C. It is possible that vaccines against certain other arbor-borne viruses can be prepared using the technique similar to this one.

Proposed course:

Studies of vaccines for the prevention of CTF will be continued and expanded, and further effort will be made to apply the vaccine to the human population. Much of this work will be done in cooperation with Dr. Gordon Meiklejohn of the University of Colorado. Attempts to study the presence and distribution of virus in ticks will be continued. Special emphasis will be placed upon the use of fluorescent antibody techniques in this study. Continued effort will be made to describe further the clinical aspects of CTF and to determine the factors responsible for the severe bleeding tendencies often encountered which result in a fatal outcome.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Burgdorfer, Willy: Colorado tick fever. The behavior of CTF virus in the porcupine. J. Inf. Dis. 104(1): 101-104. Jan.-Feb. 1959.

Rozeboom, Lloyd E. and Burgdorfer, Willy: Development of Colorado tick fever virus in the Rocky Mountain wood tick, Dermacentor andersoni. Am. J. Hyg. 69(2): 138-145. Mar. 1959.

Burgdorfer, Willy and Eklund, Carl M.: Studies on the ecology of Colorado tick fever virus in western Montana. Am. J. Hyg. 69(2): 127-137. Mar. 1959.

Gerloff, Robert K. and Eklund, Carl M.: A tissue culture neutralization test for Colorado tick fever antibody and use of the test for serologic surveys. J. Inf. Dis. 104(2): 174-183. Mar.-Apr. 1959.

Gerloff, Robert K. and Larson, Carl L.: Experimental infection of rhesus monkeys with Colorado tick fever virus. Am. J. Path. 35(5): 1043-1054. Sept.-Oct. 1959.

In press:

Eklund, Carl M., Kohls, Glen M., Jellison, William L., Burgdorfer, Willy, Kennedy, Richard C., and Thomas, Leo A.: The clinical and ecological aspects of Colorado tick fever. Proc. Intern. Cong. Trop. Med. and Malaria, Lisbon, Sept. 1958.

December 11, 1959

To: Dr. Justin Andrews, Director
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Bethesda 14, Maryland

From: Carl L. Larson, Director
Rocky Mountain Laboratory

Subject: RML Publications for the period January through November 1959

1. Salvin, S. B.: The influence of leucocytes from sensitized mice on resistance to Histoplasma capsulatum. Am. J. Hyg. 68(3): 233-241. Nov. 1958.

2. Hughes, Lyndahl E. and Philip, Cornelius B.: Experimental tick paralysis in laboratory animals and native Montana rodents. Proc. Soc. Exp. Biol. & Med. 99(2): 316-319. Nov. 1958.

3. Jellison, William L., Stoenner, Herbert G., and Berg, George M.: Leptospirosis among Indians in the Dakotas. Rocky Mtn. Med. Jour. 55(12): 56-58. Dec. 1958.

4. Brennan, James M.: A Croesus among insects. Nat. History 68(1): 56. Jan. 1959.

5. Philip, Cornelius B.: New records of Tabanidae in the Antilles. Supplemental report. Am. Mus. Novitates No. 1921: 1-7. Dec. 1958.

6. Jellison, William L., Bell, J. Frederick, Vertrees, J. D., Holmes, M. A., Larson, Carl L., and Owen, Cora R.: Preliminary observations on diseases in the 1957-58 outbreak of Microtus in western United States. Trans. 23rd N. A. Wildlife Conf., pp. 137-145. Mar. 1958.

7. Thomas, Leo A., Eklund, Carl M., and Rush, William A.: Susceptibility of garter snakes (Thamnophis spp.) to western equine encephalomyelitis virus. Proc. Soc. Exp. Biol. & Med. 99(3): 698-700. Dec. 1958.

8. Philip, Cornelius B. and Aitken, Thomas H. G.: Records of Tabanidae from Sardinia and Corsica. Memorie della Societa Entomologica Italiana 37: 87-97. Nov. 1958.

9. Rush, William A., Brennan, James M., and Eklund, Carl M.: A natural hibernation site of the mosquito, Culex tarsalis Coquillett, in the Columbia River Basin, Washington. Mosq. News 18(4): 288-293. Dec. 1958.

10. Eklund, Carl M., Hadlow, William J., Pickens, Edgar G., and Gerloff, Robert K.: Detecting poliovirus in vaccine lots used in Idaho in 1955. Pub. Health Rep. 74(1): 60-66. Jan. 1959.

11. Brennan, James M.: Synonymy of Odontacarus Ewing, 1929, and Acomatacarus Ewing, 1942, with redescriptions of O. dentatus (Ewing) and O. australis (Ewing), also descriptions of three new species from southern United States (Acarina: Trombiculidae). Ann. Ent. Soc. Amer. 52(1): 1-6. Jan. 1959.

12. Brennan, James M. and Jones, Eleanor K.: Keys to the chiggers of North America with synonymic notes and descriptions of two new genera (Acarina: Trombiculidae). Ann. Ent. Soc. Amer. 52(1): 7-16. Jan. 1959.

13. Ribi, Edgar, Milner, Kelsey C., and Perrine, Theodore D.: Endotoxic and antigenic fractions from the cell wall of Salmonella enteritidis. Methods for separation and some biological activities. J. Immunol. 82(1): 75-84. Jan. 1959.

14. Jellison, W. L., Epler, Deane C., Kuhns, Edith, and Kohls, Glen M.: Tularemia in man from a domestic rural water supply. Arquivos V Congresso Internacional de Microbiologia, Rio de Janeiro, Aug. 17-24, 1950. 1: 301-304. 1958.

15. Philip, Cornelius B., Hughes, Lyndahl E., Rao, K. N. A., and Kalra, S. Lal: Studies on Indian tick typhus and its relation to other human, typhuslike rickettsioses. Arquivos V Congresso Internacional de Microbiologia, Rio de Janeiro, Aug. 17-24, 1950. 1: 571-574. 1958.

16. Lackman, David, Casey, Mary, Philip, Robert, Owen, Cora, and Reinhard, Karl: A comparison of influenza in the northwestern United States caused by A-prime and Asian influenza viruses. Can. J. Pub. Health 50(2): 71-79. Feb. 1959.

17. Gerloff, Robert K., Larson, Carl L., and Glesne, Lillian R. B.: Naturally occurring poliomyelitis antibody in Hamilton, Montana. Pub. Health Rep. 74(2): 175-180. Feb. 1959.

18. Jellison, William L.: Fleas and disease. Ann. Rev. Ent. 4: 389-414. 1959.

19. Burgdorfer, Willy: Colorado tick fever. The behavior of CTF virus in the porcupine. J. Inf. Dis. 104(1): 101-104. Jan.-Feb. 1959.

20. Brennan, James M. and Loomis, Richard B.: A review of the reptile chiggers, genus Fonsecia (Acarina, Trombiculidae), with descriptions of two new American species. J. Parasitol. 45(1): 53-64. Feb. 1959.

21. Ribi, Edgar, Perrine, Theodore, List, Robert, Brown, William, and Goode, Granville: Use of pressure cell to prepare cell walls from mycobacteria. Proc. Soc. Exp. Biol. & Med. 100(3): 647-649. Mar. 1959.

22. Philip, R. N., Reinhard, K. R., and Lackman, D. B.: Observations of a mumps epidemic in a "virgin" population. Am. J. Hyg. 69(2): 91-111. Mar. 1959.

23. Burgdorfer, Willy and Eklund, Carl M.: Studies on the ecology of Colorado tick fever virus in western Montana. Am. J. Hyg. 69(2): 127-137. Mar. 1959.

24. Rozeboom, Lloyd E. and Burgdorfer, Willy: Development of Colorado tick fever virus in the Rocky Mountain wood tick, Dermacentor andersoni. Am. J. Hyg. 69(2): 138-145. Mar. 1959.

25. Luoto, Lauri: The epidemiology of Q fever in the United States. Am. J. Pub. Health 49(3): 334-338. Mar. 1959.

26. Gerloff, Robert K. and Eklund, Carl M.: A tissue culture neutralization test for Colorado tick fever antibody and use of the test for serologic surveys. J. Inf. Dis. 104(2): 174-183. Mar.-Apr. 1959.

27. Hoyer, Bill H., Bolton, Ellis T., Ritter, Daniel B., and Ribi, Edgar: Simple method for preparation of purified, radioactive poliovirus particles: electron micrograph. Virology (Notes) 7(4): 462-464. Apr. 1959.

28. Salvin, S. B. and Smith, Robert F.: Delayed hypersensitivity in the development of circulating antibody. The effect of X-irradiation. J. Exp. Med. 109(4): 325-338. Apr. 1959.

29. Bell, J. Frederick, Jellison, William L., Owen, Cora R., and Larson, Carl L.: Applicability of the Ascoli test to epizootic tularemia in wild rodents. J. Wildlife Mgmt. 23(2): 238-240. Apr. 1959.

30. Philip, Cornelius B.: Philippine Zoological Expedition 1946-1947. Tabanidae (Diptera). Fieldiana: Zoology 33: 543-625. Apr. 1959.

31. Salvin, Samuel B.: Delayed-type hypersensitivity and antibody formation--a discussion. In Mechanisms of Hypersensitivity, Little, Brown & Co., Boston, Mass. pp. 450-452. 1959.

32. Bell, J. Frederick: Transmission of rabies to laboratory animals by bite of a naturally infected insectivorous bat. Science 129(3361): 1490-1491. May 1959.

33. Stoenner, Herbert G., Jellison, William L., Lackman, David B., Brock, Darrell, and Casey, Mary: Q fever in Idaho. Am. J. Hyg. 69(3): 202-213. May 1959.

34. Casey, Mary L. and Smith, Bettie: A family outbreak of Shigellosis. Rocky Mtn. Med. Jour. 56(7): 33-35 and 46. July 1959.

35. Bell, J. Frederick and Meis, Armon: Pericarditis in infection due to Coxsackie virus group B, type 3. New Eng. J. Med. 261(3): 126-128. July 1959.

36. Salvin, S. B. and Smith, R. F.: Antigens from the yeast phase of Histoplasma capsulatum. III. Isolation, properties, and activity of a protein-carbohydrate complex. J. Inf. Dis. 105(1): 45-53. July-Aug. 1959.

37. Bell, J. Frederick and Hadlow, W. J.: Responses of newborn and adult domestic rabbits (Oryctolagus) to infections with Coxsackie viruses. J. Inf. Dis. 105(1): 54-60. July-Aug. 1959.

38. Salvin, S. B.: Current concepts of diagnostic serology and skin hypersensitivity in the mycoses. Am. J. Med. 27(1): 97-114. July 1959.

39. Philip, Cornelius B.: Some epidemiological considerations in Rocky Mountain spotted fever. Pub. Health Rep. 74(7): 595-600. July 1959.

40. Hoogstraal, Harry and Kohls, Glen M.: The Haemaphysalis ticks (Acarina: Ixodidae) of birds. I. H. ornithophila n. sp. from Burma and Thailand. J. Parasitol. 45(4): 417-420. Aug. 1959.

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43. Philip, Robert N., Weeks, Willard T., Reinhard, Karl R., Lackman, David B., and French, Carolyn: Observations on Asian influenza on two Alaskan Islands. Pub. Health Rep. 74(8): 737-745. Aug. 1959.

44. Collins, A. Patrick: Library of the Rocky Mountain Laboratory. Stechert-Hafner Book News, Stechert-Hafner, Inc. & Hafner Publ. Co. New York. 14(1): 1-2. Sept. 1959.

45. Gerloff, Robert K. and Larson, Carl L.: Experimental infection of rhesus monkeys with Colorado tick fever virus. Am. J. Path. 35(5): 1043-1054. Sept.-Oct. 1959.

46. Stoenner, Herbert G., Holdenried, Robert, Lackman, David, and Orsborn, John S., Jr.: The occurrence of Coxiella burnetii, Brucella, and other pathogens among fauna of the Great Salt Lake desert in Utah. Am. J. Trop. Med. & Hyg. 8(5): 590-596. Sept. 1959.

47. Philip, Cornelius B.: New North American Tabanidae. X. Notes on synonymy, and description of a new species of Chrysops. Trans. Am. Ent. Soc. 85: 193-217. Oct. 1959.

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49. Philip, Cornelius B.: Some records of Tabanidae (Dipt.) from Iran. Mitteilungen der Schweizerischen Entomologischen Gesellschaft XXXII (2 & 3): 333-336. Oct. 1959.

In press:

1. Bell, J. Frederick and Moore, G. John: Rabies virus isolated from brown fat of naturally infected bats. Proc. Soc. Exp. Biol. & Med.

2. Brennan, James M. and White, Jesse S.: New records and descriptions of chiggers (Acarina: Trombiculidae) on bats in Alabama. J. Parasit.

3. Brennan, James M.: Ectonyx, a new neotropical genus of chiggers (Acarina: Trombiculidae). Acarologia.

4. Bell, E. John and Stoenner, Herbert G.: Immunologic relationships among the spotted fever group of rickettsias determined by toxin neutralization tests in mice with convalescent animals serums. J. Immunol.

5. Brennan, James M. and Dalmat, Herbert C.: Chiggers of Guatemala (Acarina: Trombiculidae). Ann. Ent. Soc. Amer.

6. Eklund, Carl M., Kohls, Glen M., Jellison, William L., Burgdorfer, Willy, Kennedy, Richard C., and Thomas, Leo A.: The clinical and ecological aspects of Colorado tick fever. Proc. Intern. Cong. Trop. Med. and Malaria, Lisbon, Sept. 1958.

7. Jellison, William L.: Sodoku. Rat-bite fever due to Spirillum minus Carter. Diagnostic Procedures and Reagents, edited by Dr. William B. Cherry, 4th Edition.

8. Kohls, Glen M.: Records and new synonymy of New World Haemaphysalis ticks, with descriptions of the nymph and larva of H. juxtakochi Cooley. J. Parasit.

9. Kohls, Glen M.: Ixodides. Encyclopedia of Science and Technology, McGraw-Hill Book Co.

10. Lackman, David B., Bell, J. Frederick, Larson, Carl L., Casey, Mary L., and Benson, W. W.: An intradermal sensitivity test for Q fever in man. Arch. Pasteur Inst., Tunis.

11. Lackman, David, Gerloff, Robert, and Philip, Robert: A comparison of complement fixation and metabolism inhibition as methods of evaluating poliomyelitis antibody response. Am. J. Pub. Health.

12. Larson, Carl L., Ribi, Edgar, Milner, Kelsey C., and Lieberman, Jacob E.: A method for titrating endotoxic activity in the skin of rabbits. J. Exp. Med.

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17. Philip, Cornelius B.: Malaysian Parasites. XXXV. Description of some Tabanidae (Diptera) from the Far East. Malaysian Parasites.

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24. Salvin, S. B. and Smith, R. F.: Delayed hypersensitivity and the anamnestic response. J. Immunol.

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27. Ribi, Edgar, Hoyer, Bill H., Milner, Kelsey C., Perrine, Theodore D., Larson, Carl L., and Goode, Granville: Physical and chemical analysis of endotoxin from Salmonella enteritidis. J. Immunol.

28. Burgdorfer, Willy: The possible role of ticks as vectors of Leptospirae. II. Infection of the ixodid ticks, Dermacentor andersoni and Amblyomma maculatum, with Leptospira pomona. Exp. Parasit.

29. Thomas, Leo A. and Smith, James V.: The 1958 encephalitis outbreak in northern Utah: 2. Infection rates in birds, mammals, and mosquitoes. Mosq. News.

SUMMARY OF ACTIVITIES
LABORATORY OF BACTERIAL DISEASES
December 18, 1959

The research program of the Laboratory of Bacterial Diseases has continued in the same general areas as last year with some change in emphasis to be noted.

The staphylococcal studies have been directed almost entirely toward development and standardization of tests for use in determination of relative pathogenicity of staphylococcal strains since such base line measurements are essential to further basic studies on the several factors determining pathogenicity of a particular strain. With such a complex organism no single test will serve to characterize the potentials of a strain. It appears that tests determining minimal killing dose (for the mouse), minimal infective dose, and toxic activity, employed in conjunction may serve this purpose. These studies are progressing slowly but soundly.

In Dr. Verder's special fields of interest, due to lack of time because of the initiation of the staphylococcal project, little has been done on the Pseudomonas and PPLO projects. However, a collaborative project on Pseudomonas with the Laboratory of Pharmacology and Toxicology, NIAMD, is being developed. And it is of note that L forms of several staphylococcus strains have been produced. Basic studies of these forms will be undertaken when the time of personnel permits.

While both bovine and human brucellosis in the United States are decreasing, world-wide brucellosis is one of the major infectious diseases with tremendous impact on agricultural economics as well as a significant cause of human illness. We continue to collaborate with other brucellosis centers in problems relative to diagnostic tests and taxonomy. Due to great confusion in the characterization of Brucella species, last spring a Subcommittee on Taxonomy of Brucella, of the International Committee on Bacteriological Nomenclature was appointed to consider this genus. The Chief, Laboratory of Bacterial Diseases is a member of this Committee. The collaborative studies with members of the Veterinary Science Department of Purdue University on experimental swine brucellosis have been published. The major laboratory project still continuing is that on antigenic structure of Brucella. Some further projected studies are described in the Brucellosis Project Report.

In line with the basic research interests of this Laboratory an increasing amount of the effort previously devoted to studies on brucellosis is being shifted to a new project on intracellular parasitism.

Essentially these studies deal with the host-parasite relationship at the cellular level employing the basic techniques of enzymology, biochemistry, and physical biology. It is anticipated that this shift in emphasis will continue to increase.

There are other areas within the province of this Laboratory which should be developed were space, personnel, and financial support available. Among these are the diarrheal diseases and the basic field of PPLO and L form research.

During the year the Laboratory lost a scientist, Dr. Grace A. Beal, by death. A suitable replacement has not yet been found.

There remains a continuing need for certain additional equipment and increased monetary support to pursue the basic studies mentioned.

Serial No. NIAID-200
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Brucellosis

Principal Investigator: Dr. Norman B. McCullough

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 2-1/2
Professional: 1/2
Other: 2

Project Description:

Objectives:

The objectives of this project are broad and studies are conducted on all aspects of brucellosis, including the nutrition, metabolism, and enzymatic make-up of the organism, mechanism of action of antibiotics on the organism, factors determining virulence of the organism, the development and efficacy of prophylactic immunizing agents, field studies to determine the occupational hazard of the disease, epidemiology, diagnosis and therapy of the disease in man.

Clinical studies concern diagnostic criteria for the disease in man, especially in borderline cases of chronic illness in which present cultural and serological techniques fail to provide reliable evidence of infection; studies of the pathogenesis of the disease; and the evaluation of current therapeutic regimens.

Part B included

Methods Employed:

The Warburg Technique is employed in enzymatic and metabolic studies; growth in a synthetic medium is used for nutrition experiments; guinea pig inoculation for virulence and vaccine studies; occupationally exposed groups for epidemiological studies; human patients for studies on diagnosis and therapy.

Major Findings:

Collaborative studies with members of the Veterinary Science Department of Purdue University on swine brucellosis have been completed and the results published in two papers appearing in the American Journal of Veterinary Research. In one of these studies it was shown that Brucella neotomae is pathogenic for swine and produces a disease which is indistinguishable from that due to Br. suis. Extensive laboratory studies of this organism suggest that it is a variant of Br. suis and should not be accorded species rank. It differs from classical Br. suis in color value of the colony as viewed with oblique lighting and in having a slightly greater sensitivity to the dye thionin. With the progress attained in the control program for bovine brucellosis, knowledge of additional reservoirs of Brucella infection and of the behavior and potentialities of aberrant strains in domestic animals assume increasing importance.

The study of antigenic relationships among the Brucella has progressed and the findings are being checked for universality on representative series of cultures from various parts of the world If the results of this survey continue to confirm our previous findings they should indicate that the use of so-called monospecific sera in the identification of Brucella species should be discontinued, or the procedure greatly modified. Most of the disagreement between various centers in identification of Brucella species hinges upon the results obtained with the current method of immunological typing.

Significance to the Program of the Institute:

In terms of world-wide significance, brucellosis ranks in incidence and importance with malaria, tuberculosis, and parasitic diseases, in relation to the welfare of mankind. Even in the United States, by reported incidence, it is the most important disease of animals transmissible to man, is the cause of serious economic loss to the livestock industry, is an occupational disease, and one which presents many problems in the diagnosis and therapy of the infection in man. The organism readily lends itself to basic studies outlined elsewhere in this report.

Proposed Course of Project:

Continue studies under way.

Continue to collaborate with other Brucella research centers throughout the world in the development and standardization of laboratory tests for species identification, and for diagnosis of Brucella infection.

Study of Brucella bacteriophage as regards basic phenomena of bacteriophagy and potential use of phage for preparation of enzymatic and subcellular fractions of Brucella for biochemical studies, and as possible immunizing agents. Investigate the utility of specific phages as an aid in identification of Brucella species and strains.

Continue to use Brucella as a basic tool in the study of mechanism of development of acquired resistance to antibiotics and in studies of intracellular parasitism.

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

(1) McCullough, N. B. and Beal, G. A.: La Estabilidad Biologica Del Genero Brucella. Boletin de la Oficina Sanitaria Panamericana XLVI: 213-221, Marzo, 1959.

(2) Beal, G. A.; Taylor, D. B.; McCullough, N. B.; Claflin, R. M.; and Hutchings, L. M.: Experimental Infection of Swine with Brucella Melitensis (Mediterranean Type). Am. J. Vet. Res. 20: 634-641, July, 1959.

(3) Beal, G. A.; Lewis, R. E.; McCullough, N. B.; and Claflin, R. M.: Experimental Infection of Swine with Brucella Neotomae. Am. J. Vet. Res. 20: 872-875, Sept. 1959.

Honors and Awards relating to this project:

Principal investigator invited to serve as a member of the Subcommittee on Taxonomy of Brucella of the International Commitee on Bacteriological Nomenclature.

Serial No. NIAID-201
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Basic Studies on Staphylococcus

Principal Investigator: Dr. Elizabeth Verder

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 1-10/12
Professional: 10/12
Other: 1

Project Description:

Objectives:

1. To develop an objective means of measuring virulence of Staphylococci.

2. To determine the factors responsible for virulence.

3. To determine the significance of such factors in antibiotic resistant strains and the relationship (if any) between the ability to develop antibiotic resistance and pathogenicity.

4. To study the mechanism of antibiotic resistance in Staphylococcus.

Part B not included.

Methods Employed:

The use of experimental animals and tissue cultures in defining overall virulence, and in study of individual fractions or products of the Staphylococcus.

Use of physical and chemical methods in fraction of the organism and its products to allow assessment of individual factors in pathogenicity and in cell injury.

Nutritional studies on the production of factors concerned in pathogenicity, and in antibiotic resistance.

Enzymatic studies, particularly those employing the Warburg technique, for determining the role of particular enzyme systems in antibiotic resistance and in pathogenicity.

The techniques of immunology, genetics, bacterial transformation, and phage induction in exploring the mechanism of development of antibiotic resistance.

Gel diffusion techniques using both tubes and plates and hemagglutination precipitin tests are being employed. An attempt is being made to correlate mouse protection properties of human sera with certain characteristics of *in vitro* reactions.

Major Findings:

All studies have dealt with properties correlated with invasiveness.

It is probable that no single test will adequately measure pathogenicity of Staphylococcus. Over the past year we have investigated and attempted to standardize techniques to determine minimal killing doses for mice as well as to determine minimal infecting doses using the kidney as the index organ for determination of infection. Quantitative recovery of staphylococci is done at death or sacrifice of animals 14 days after inoculation.

Preliminary studies indicate that staphylococcal toxic activity may be determined quantitatively by virtue of production of consolidation of the lungs of mice.

While some further work on standardization and confirmation is necessary these techniques give good promise of providing a reproducible means of determining comparative pathogenicity of Staphylococcus.

Significance to the Program of the Institute:

The Staphylococcus has become an important public health problem due to the rapid development of antibiotic resistant strains and their spread through the population. Control of this situation is dependent on the acquisition of more and extensive basic information on the Staphylococcus and on the mechanism of antibiotic resistance. The problem of antibiotic resistance is not limited to the Staphylococcus but is currently becoming of importance in other groups of organisms. Indeed, the development of resistance to external agents is a fundamental phenomenon in biology and deserving of intensive study. The currently described project is designed to provide basic knowledge in regard to the Staphylococcus in particular, but also concerns the broad problem of induced resistance in general.

Proposed Course of Project:

As described above.

Serial No. NIAID-202
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Identification of Pseudomonas

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: None

Cooperating Units: Dr. Millikin, NIAMD

Man Years (calendar year 1959)
Total: 1/12
Professional: 1/12
Other: 0

Project Description:

Objectives:

a) Identification of strains for other scientists.

b) Orientation of scientists in methods used for study of biochemical characteristics of various species and for serological typing.

c) Distribution of cultures of strains of various species and of representative serological types of Ps. aeruginosa on request to research scientists.

d) To determine whether therapeutic antisera contains identifiable group specific antibody.

Part B not included.

Methods Employed:

The methods which we have employed are ones developed or modified in the course of our studies on the classification of Pseudomonas; the studies were initiated during work on our project on diarrheal disease of the newborn and have been carried on for eight years. A proposed scheme for the antigenic analysis of strains of Ps. aeruginosa was worked out and has been employed successfully in identifying strains by serological typing.

Dr. Millikin has succeeded in guiding a commercial biological company to the production of an antiserum that has been used successfully in the treatment of children with Ps. aeruginosa infections. He has asked us to collaborate in studies on protection of animals infected with various serological types to determine whether or not a group specific antibody is responsible for these protective properties.

Standard antigen-antibody techniques will be employed in the study. Gel diffusion studies will be done with various gamma globulin and serum preparations and several antigenic fractions of Pseudomonas.

Major Findings:

Methods have been established for use in the identification of individual strains of Pseudomonas so that epidemiological surveys in relation to the spread of resistant strains may be carried out successfully.

Significance to the Program of the Institute:

Methods have been developed for studies on the spread of a group of antibiotic resistant organisms of medical importance, particularly in infections in individuals with minimal resistance such as newborns, the aged, children with leukemia, individuals with extensive or deep burns, etc. With the increasing importance of Pseudomonas infections the findings in these studies are receiving recognition in clinical applications.

Proposed Course of Project:

Collaborative studies with Dr. Millikin as outlined above.

Serial No. NIAID-203
1. Bacterial Diseases
2. None
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Bacteria Characterized by the Production of Reproductive Filterable Granules

Principal Investigator: Elizabeth Verder, Ph.D.

Other Investigator: Dr. Alexis Shelokov was co-investigator on the project at time the organisms were isolated and any reports will be made jointly.

Cooperating Units: None

Man Years (calendar year 1959)
Total: 1/12
Professional: 1/12
Other: 0

Project Description:

Objectives:

To learn more about 1) the classification of bacteria capable of producing reproductive filterable granules, 2) the environmental changes that induce the formation of granules, 3) the potentialities of these incomplete filterable organisms in the establishment of infectious processes and 4) the factors influencing the development of more complete cells from filtered particles.

Part B not included.

Methods Employed:

Through the use of special media, organisms of this type have been isolated from the peripheral blood of several patients with illnesses with an indefinite diagnosis. Methods commonly employed in the study of organisms producing L bodies and the pleuro-pneumonia-like (PPLO) group were utilized.

Major Findings:

More detailed studies must be carried out before significant findings on these organisms may be reported. During the past year attempts to produce L forms of several strains of staphylococci have been successful. No detailed physiological studies have yet been carried out.

Significance to the Program of the Institute:

Organisms with similar characteristics have been recognized as important etiological agents of animal disease for many years. Their presence in extracts of tissue and blood serum collected from infected embryo or animals and used inadvertently in the preparation of tissue culture media has increased interest in the development of more satisfactory methods for their detection and identification. Their importance in infectious processes in man has not been adequately assessed. Since several species of Bacteriolides are present in large numbers in the intestinal canal of man and animals, some of these organisms may belong to closely related species possessing filterable granules under certain circumstances and be present in the peripheral blood frequently. The importance of the group to medical scientists is emphasized by the three day conference sponsored by the N. Y. Academy of Sciences held in January, 1959, for discussion of various phases of studies on this group of organisms; Drs. Klieneberger-Nobel and Edwards of London and Dr. Freund of Copenhagen were among the invited participants.

Proposed Course of Project:

No additional work will be carried on with these organisms until the studies on the staphylococcus have been well established.

[illegible]

[illegible]
Intramural Research Report
[illegible]

Part A

Project Title: Intracellular Parasitism

Principal Investigator: Norman E. McCullough M. D.

Other Investigators: None

Cooperating Units: None

Man Years allocated for [illegible]:
Total: 1-1/2
Professional: 1
Other: 1/2

Project Description:

Objectives:

To study host-parasite relationships in intracellular parasitism for the purpose of increasing our understanding of normal and neoplasia.

1. To determine the effect of infectious organisms on the metabolism of the infected cell.

2. To determine the effect of various bacterial products such as streptococcal toxin and bacterial fractions on cell growth and on tissue culture, and upon certain [illegible]

3. To determine the contribution of cell and tissue [illegible] antibody, antigen and enzyme systems [illegible] and their effects on the [illegible]

Part B or C Methods

4. To study whether differences can be demonstrated, in the areas mentioned above, between normal and immune cells.

Methods Employed:

The study will employ various pathogenic agents such as Brucella, Staphylococcus, pasteurella tularense, and certain viruses. It will entail the use of standard tissue culture techniques, chemically defined media for bacteria, the Warburg technique, standard chemical procedures, phase microscopy, fluorescent antibody technique, vital dyes, electrophoresis, and chromatography.

Major Findings:

New project.

Significance to the Program of the Institute:

This study concerns the basic interactions between host cell and infecting agent and hence any contribution to knowledge would be of significance to the entire field of infectious diseases and to the program of the Institute.

Proposed Course of Project:

As stated above.

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